TOSOH

CHROMATOGRAPHY CATALOG

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TOSOH HISTORY

1935	Founding of Toyo Soda Manufacturing Co., Ltd.
1936	Operation of Nanyo Manufacturing Complex begins
1971	First TSKgel GPC column developed
1974	HPLC Column Plant starts production
1977	First silica based TSKgel SW column for protein analysis
1979	Tosoh develops TOYOPEARL media for preparative chromatography
1987	Introduction of TSKgel G3000SW$_{XL}$ column, the gold standard for aggregation analysis
1993	First TSKgel Semi Micro GPC columns increase sensitivity, save time and solvent
1995	Tosoh Nanyo Gel Factory receives ISO 9001
2015	TSKgel UP-SW3000 columns for easy transfer of HPLC methods to UHPLC
2016	Protein A column for fast mAb titer determination
2017	Construction of a new R&D laboratory center announced
2019	Launch of TSKgel IIIA-NPR FcR Affinity Column or fast assessment of mAb ADCC activity which was awarded one of the Pittcon Today Excellence Awards for ingenuity and creativity in scientific advancement

NOMENCLATURE

What's in our names?

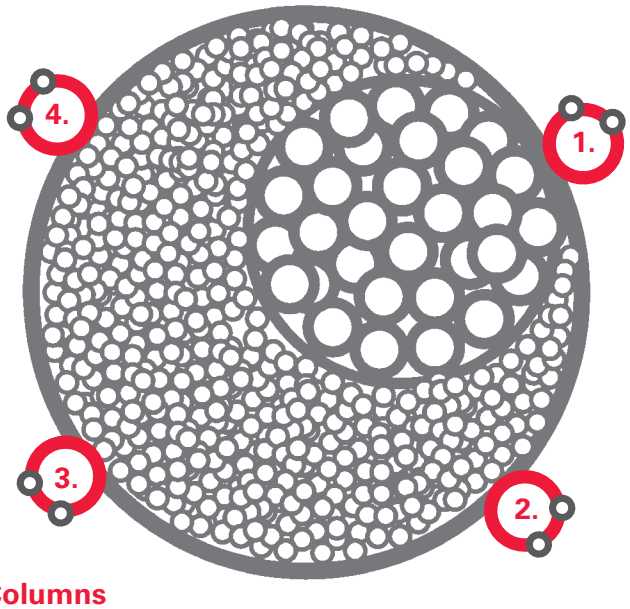
Tosoh Bioscience has the most comprehensive selection of process media resins, with a variety of pore and particle size combinations for several modes of chromatography. Here's how you can identify the right column for your analysis:

1. Stationary Phases

Tosoh Bioscience basically uses two base materials for the (U)HPLC columns: silica and polymer. Abbreviations used for the base matrix are SW for silica and PW for polymer. Stationary phases used with organic mobile phases for Gel Permeation Chromatography (GPC) consist of a styrene-divenylbenzene polymer and typically carry an 'H' in their names.

4. Additional Abbreviations

We use the following abbreviations to highlight their features:	
NPR	non-porous
HTP	High Throughput
HR	High Resolution
AF	Affinity
RP	Reversed Phase



3. Pore Size of SW-Series Columns

Grade	Pore Size SW Series (nm)
G2000, SuperSW2000	12,5
G3000, SuperSW3000, SuperSW mAb	25
UltraSW Aggregate	30
G4000	45

2. (U)HPLC Stationary Phase Ligands

TSKgel ligands	
Mode	Ligand
HILIC	Amide, NH_2
Anion Exchange	Q, DEAE
Cation Exchange	CM, SP
HIC	Ether, Phenyl, Butyl
Reversed Phase	CN, C1, C4, Phenyl, C8, C18
Affinity	Fc gamma IIIa receptor, Protein A, Boronate, Chelate, Tresyl

TABLE OF CONTENTS

INTRODUCTION
ABOUT US

WITH A GLOBAL PERSPECTIVE.

Tosoh Bioscience GmbH, a member of the Tosoh Group, markets and supports liquid chromatography solutions. Our product portfolio encompasses a comprehensive line of process media and pre-packed HPLC columns for all modes of liquid chromatography and GPC instruments. We are the only supplier of consumable chromatography solutions in the biopharmaceutical market to offer expertise for all liquid chromatography solutions, from early stage discovery through clinical trials to large-scale production. With a long history and extensive experience in chromatography, Tosoh Bioscience is more than a provider of analytical (U)HPLC columns, GPC equipment and process resins – we have a proven track record of sound scientific knowledge and technical support to our customers.

INTRODUCTION
ABOUT US

PRODUCTION

Tosoh's state of the art manufacturing sites in Japan provide products to the sales and support network across the world. The instruments, columns and media are manufactured at Tosoh's Nanyo Complex in the Yamaguchi prefecture at the southwestern tip of the mainland of Japan. All chromatography products are shipped from this ISO 13485/9001 registered facility. The Nanyo manufacturing complex is a self-contained city with its own power generation plants and port. It is a model of environmental responsibility and has earned ISO 14001 certification for environmental management.

SUPPLY CHAIN

The Bioscience Division of Tosoh Corporation is headquartered in Tokyo, Japan. Tosoh Bioscience Separations in Griesheim, Germany houses all sales, marketing and technical support activities for the separation products. The Tosoh Bioscience customer service center is located in Tessenderlo, Belgium. In Tessenderlo we inventory an extensive line of TSKgel® (U)HPLC columns and Process development columns. TOYOPEARL® and TSKgel PW bulk resin products are also inventoried at Tessenderlo in quantities suitable for resin screening or early GMP production. Larger volumes of our process resins are inventoried at the Tosoh Bioscience manufacturing site in Japan.

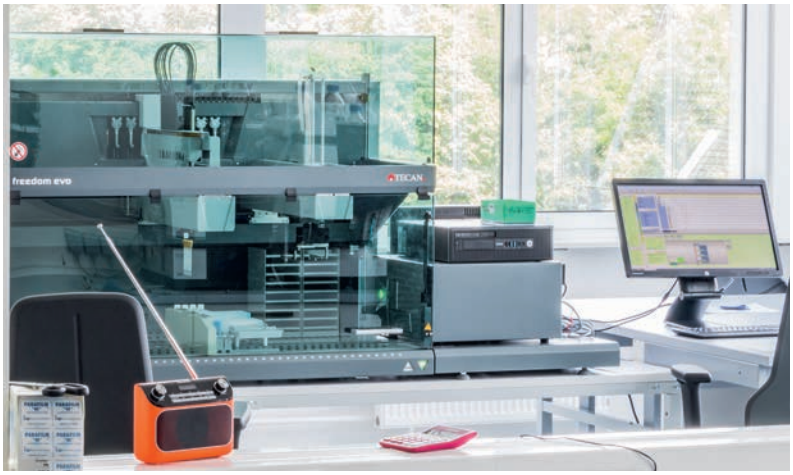
REGULATORY SUPPORT

In preparation for a filing of a new drug with the regulatory agencies it may be advisable to initiate a more detailed discussion about Tosoh Bioscience's products. Tosoh Bioscience recommends establishing a Confidential Information Disclosure Agreement (CIDA).

Tosoh Bioscience maintains Regulatory Support Files (RSF) on most of our process scale media. The file contains detailed information that describes the synthesis and quality control of our manufacturing process. In order to support your application for a new drug, please contact us through your Sales Representative.

INTRODUCTION
ABOUT US

TECHNICAL SUPPORT



Tosoh Bioscience offers a range of Technical Support services to our TSKgel, ToyoScreen, and TOYOPEARL chromatography products and EcoSEC® GPC instruments. We are committed to providing prompt and skilled service for these and other requests: to provide you with the right advice to select the best column, resin, or instrument for your application, to help you with product installation, method development, and troubleshooting, to guide you with packing TOYOPEARL and TSKgel resins into large production columns, to support you with regulatory files for a submission to the FDA.

One of the services that stand out in the industry is the Tosoh Chromatography Workshop Series providing a comprehensive background to the chromatographic purification of biomolecules. These courses provide a balance of effective presentations and practical hands-on experience under the guidance of qualified tutors.

TOSOH'S TECHNOLOGY

For over forty years our parent, Tosoh Corporation, has been a world leader in the analysis and purification of proteins. A thorough understanding of the role played by pore diameter and molecular size in chromatographic separations allows Tosoh to design higher performance resins for size exclusion, ion exchange, hydrophobic interaction, mixed mode and affinity applications.

From the research laboratory to full scale manufacturing, we offer the same polymer chemistries in our TSKgel and TOYOPEARL products. Whether you are scaling up from a TSKgel column HPLC method to TOYOPEARL resin for manufacturing, or are scaling down from TOYOPEARL resin based purification to the corresponding TSKgel column for the QC of your target, we make it easy to develop methods to do both.

INTRODUCTION
PRODUCT LINES

TSKgel COLUMNS

Our TSKgel columns for (U)HPLC are used for the analysis and purification of proteins, peptides, biopolymers and low molecular weight compounds. We provide (U)HPLC columns for many chromatographic modes such as hydrophobic/hydrophilic interaction, ion exchange, reversed phase, and affinity chromatography. Our core competency is the manufacturing of size exclusion columns for the analysis of proteins. For over 30 years TSKgel SW-type silica-based columns have been the biopharmaceutical industry's standard in gel filtration chromatography of biomolecules.

TSKgel columns are known 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 U.S. Pharmacopoeia (see Appendix C). 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.

TSKgel RESINS

The highly cross linked polymeric resins with particle sizes of 20 µm and 30 µm used in TSKgel columns are also available in bulk quantities for large scale ion exchange and hydrophobic interaction chromatography. Their mechanical stability and permeability make them excellent for use when increased separation performance and plate count are needed for optimum preparative or process chromatography.

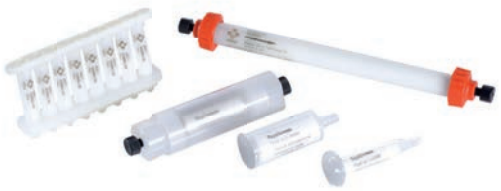
TOYOPEARL RESINS

TOYOPEARL resins are hydrophilic macroporous methacrylic resins. Their rigid polymeric backbone has better pressure-flow properties than most other stationary phases. Therefore, higher linear velocities can be used to achieve faster purification cycles. The resins are offered in many different pore diameters for size exclusion, ion exchange, hydrophobic interaction, multimodal, and affinity chromatography.

PRE-PACKED PROCESS DEVELOPMENT PRODUCTS

MiniChrom® Columns with 5 mL bed volume (8 mm ID x 10 cm L) are the most convenient tools for method development. They are available for most TOYOPEARL and some TSKgel resins.

ToyoScreen® process development columns are easy to use. They are available as 1 mL and 5 mL pre-packed cartridges. Placed in the ToyoScreen holder they can be connected to most laboratory chromatographic systems.



The most popular TOYOPEARL resins are also available in RoboColumn® format. RoboColumns are miniaturized chromatographic columns for operation with a robotic liquid handling system.

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL resins. They are available in several configurations for antibody affinity, ion exchange, HIC, and mixed-mode chromatography. Resin Seeker plates can be operated manually or in an automated high throughput screening system.

INTRODUCTION - CHROMATOGRAPHIC ANALYSIS OF BIOMOLECULES

High performance liquid chromatography (HPLC) and, increasingly, ultra-high performance liquid chromatography (UHPLC) are the analytical workhorses of the pharmaceutical industry. All stages of the product's lifecycle, from early development until production and stability testing need chromatographic analysis to characterize and quantify target molecules and impurities.

Biopharmaceuticals are the fastest growing product segment of the pharmaceutical industry and a thorough characterization of therapeutic biomolecules is key for the successful submission of data for regulatory approvals of new drugs, no matter whether biologic, biosimilar or biobetter. Quality control needs effective analytical tools for fast determination of critical quality attributes of the various kinds of biopharmaceuticals, such as monoclonal antibodies (mAbs) and other therapeutic proteins. With new biopharmaceutical formats, such as bispecific mAbs, antibody fragments and antibody-drug-conjugates (ADCs) in the pipeline, rapid and thorough characterization will become even more important.

Size exclusion chromatography (SEC) and ion exchange chromatography (IEC) are typical modes for separation of proteins in native form and are routinely used for the characterization of biotherapeutics. Especially SEC has become a Swiss-army knife for protein aggregate determination. It is a mild technique that preserves biological activity and structural integrity. It can virtually be considered a platform – quick and straightforward. Hydrophobic interaction chromatography (HIC) became a standard method for DAR analysis of ADCs. Reversed phase (RPC) and hydrophilic interaction liquid chromatography (HILIC) are used to characterize peptides or oligosaccharide chains after enzymatic cleavage. Protein A affinity chromatography allows fast determination of antibody titers in screening or process monitoring. Gel permeation chromatography (GPC) is used to characterize synthetic and natural polymers.

TSKgel UHPLC and HPLC columns are popular in the biotech and biopharmaceutical industry and are used in R&D, method development, production, quality control and stability testing.

Are you interested in learning more about the basics of chromatography? Visit us on YouTube. Tosoh Basics - What is chromatography? www.youtube.com/watch?v=2QVCxK0QPeg

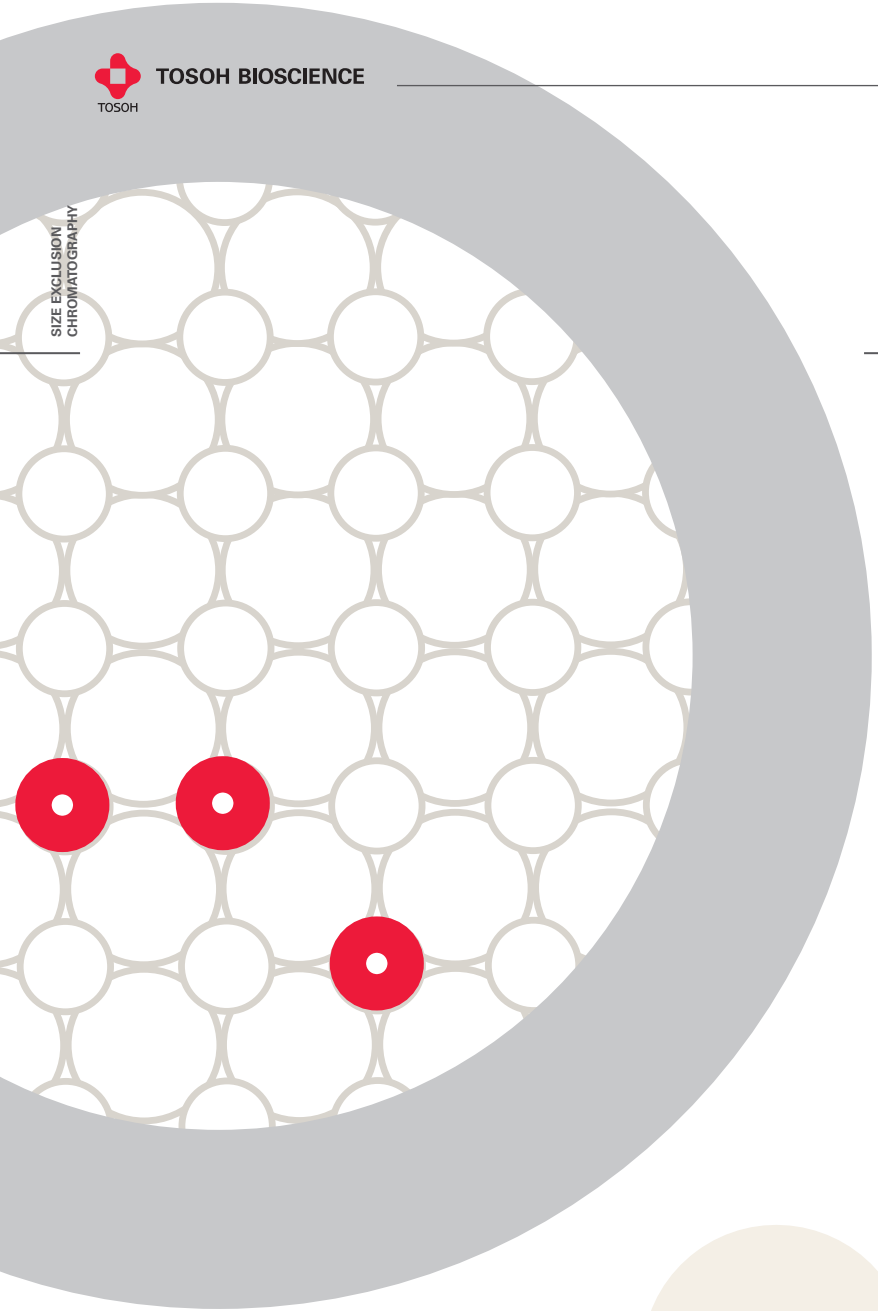
WHAT'S NEW

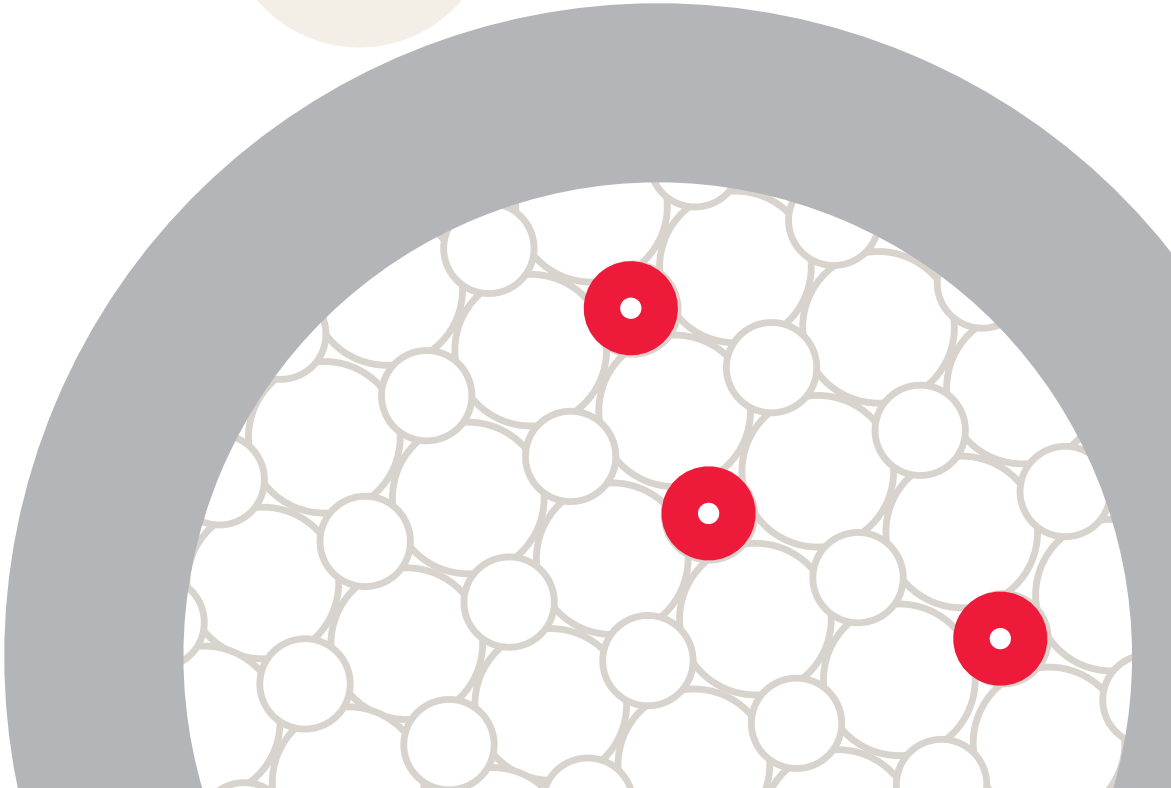
TSKgel UP-SW Series - PAGE 10

- Efficient mAb characterization by UHPLC
- High resolution size exclusion analysis of biomolecules
- Consistent lot-to-lot reproducibility and long column lifetime
- Plug and play method transfer from HPLC to UHPLC

FcR-IIIA-NPR COLUMN FOR ADCC ACTIVITY ANALYSIS- PAGE 70

- Innovative high performance affinity chromatography column
- Separates antibody glycoforms based on differences in ADCC activity
- Recombinant hFc gamma receptor IIIA ligand immobilized on NPR particle
- Fast, robust, and highly reproducible analysis





SEC
SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

TSKgel SW-type

TSKgel UP-SW
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 SuperOligoPW

TSKgel Alpha-type

TSKgel Alpha
TSKgel SuperAW
TSKgel VMpak

TSKgel H-type

TSKgel HXL
TSKgel HHR
TSKgel HHR-HT
TSKgel SuperH
TSKgel SuperHZ
TSKgel SuperMultiporeHZ
TSKgel MultiporeHXL

TSKgel SEC Standards

Tosoh is well known for offering not only process resins, but also (U)HPLC columns for the analytical separation of biomolecules in the biopharmaceutical industry.

”Although, several columns showed a comparable resolution, the Tosoh TSKgel UP-SW3000 column (2 µm, 4.6 x 30 mm) convinced us in terms of robustness, especially the high lot-to-lot stability, an absolute requirement for quality control under GMP conditions.”

Dr. Raphael Ruppert
Roche Diagnostics

SEC HIGHLIGHTS

HIGHLIGHTS TSKgel UP-SW SERIES

- UP-SW3000 - perfect fit for antibody aggregate analysis
- UP-SW2000 - perfect fit for small proteins and peptides
- Established pore characteristics enable plug and play method transfer from HPLC to UHPLC
- Excellent lot-to-lot reproducibility
- Available in two dimensions, one for high throughput the other for high resolution

HIGHLIGHTS TSKgel UltraSW Aggregate

- Designed to offer increased resolution for higher mAb aggregates
- Covers molecular weight range of antibody aggregates and high molecular weight proteins
- Adds a new pore size option to the TSKgel SW family
- Can be used with HPLC and UHPLC systems

FEATURES

- Rigid and inert hydrophilic and hydrophobic packings
- Four series with different solvent compatibility
- Broad range of pore sizes

BENEFITS

- Excellent physical strength and low adsorption
- Suitable for both types of size exclusion, aqueous (GFC) and organic (GPC)
- Perfect mass range for many applications

SIZE EXCLUSION CHROMATOGRAPHY HOW DOES IT WORK?

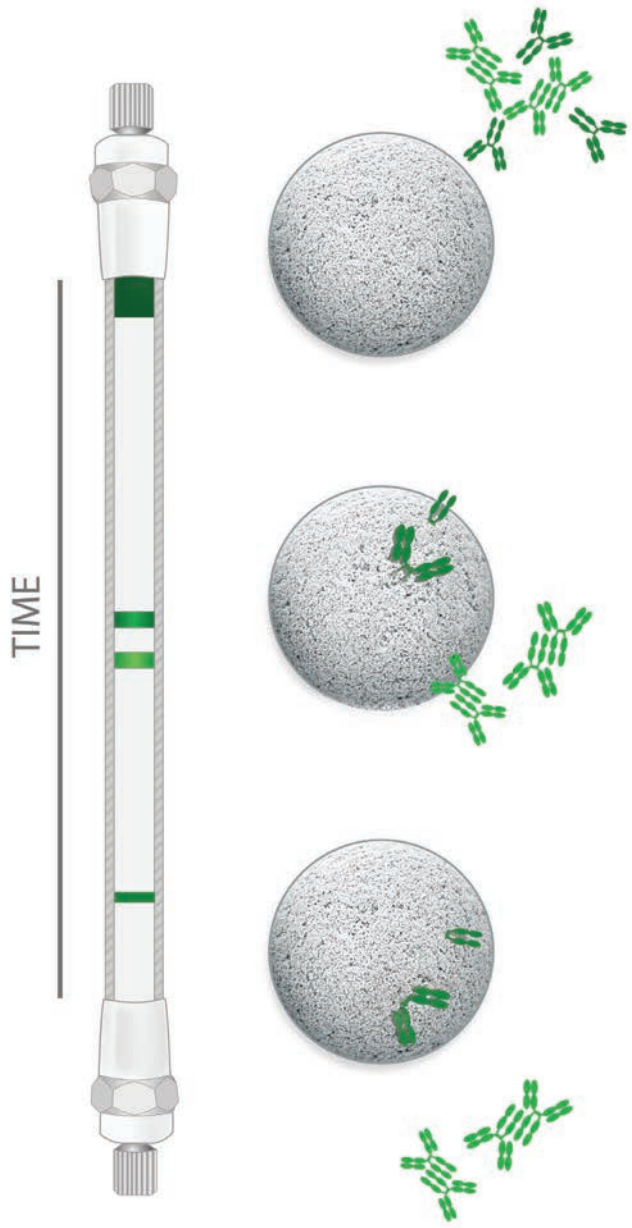
Size Exclusion Chromatography (SEC) separates molecules based on their size. It is usually applied to large molecules such as proteins or synthetic polymers. When an aqueous mobile phase is used, SEC is also referred to as gel filtration chromatography (GFC). When an organic eluent is applied, SEC is referred to as gel permeation chromatography (GPC). GPC is typically used to determine the molecular weight (MW) and the MW distribution of synthetic polymers while GFC is used to separate biopolymers based on their size.

In SEC, components of a mixture are separated according to their molecular size, or more precisely, their hydrodynamic volume, 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.

For a detailed introduction into Size Exclusion Chromatography please refer to our SEC and GPC Column Brochures.

FIGURE 1

SIZE EXCLUSION CHROMATOGRAPHY ILLUSTRATION



SEC
STATIONARY PHASES

Tosoh Corporation has a proud history of innovation in size exclusion chromatography. TSKgel SEC columns are known worldwide for their reliability and suitability for the analysis of proteins, peptides and other biological macro-molecules. The TSKgel SW, PW, Alpha/AW and H column lines consist of either silica based or polymer based packings, ranging in particle size from 2 µm to 20 µm. Columns are available in analytical through semi-preparative size, in stainless steel, PEEK or glass.

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.

TSKgel columns for GPC analysis consist of the TSKgel Alpha/SuperAW and H series column lines, which are all based on polymer base particles. 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. For the GPC analysis of organic-soluble polymers, Tosoh developed TSKgel H series, filled with polystyrene/divinylbenzene polymer particles.

TABLE I

SUMMARY OF TSKgel SIZE EXCLUSION COLUMN LINES

Column line	TSKgel SW / SWXL / SuperSW / UltraSW / UP-SW	TSKgel PW / PWXL	TSKgel Alpha / TSKgel SuperAW	TSKgel H
Particle composition	Silica	Polymethacrylate	Highly crosslinked polymethacrylate	PS-DVB
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
Application focus	proteins	water soluble polymers	intermediate polar polymers	organic-soluble polymers

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.

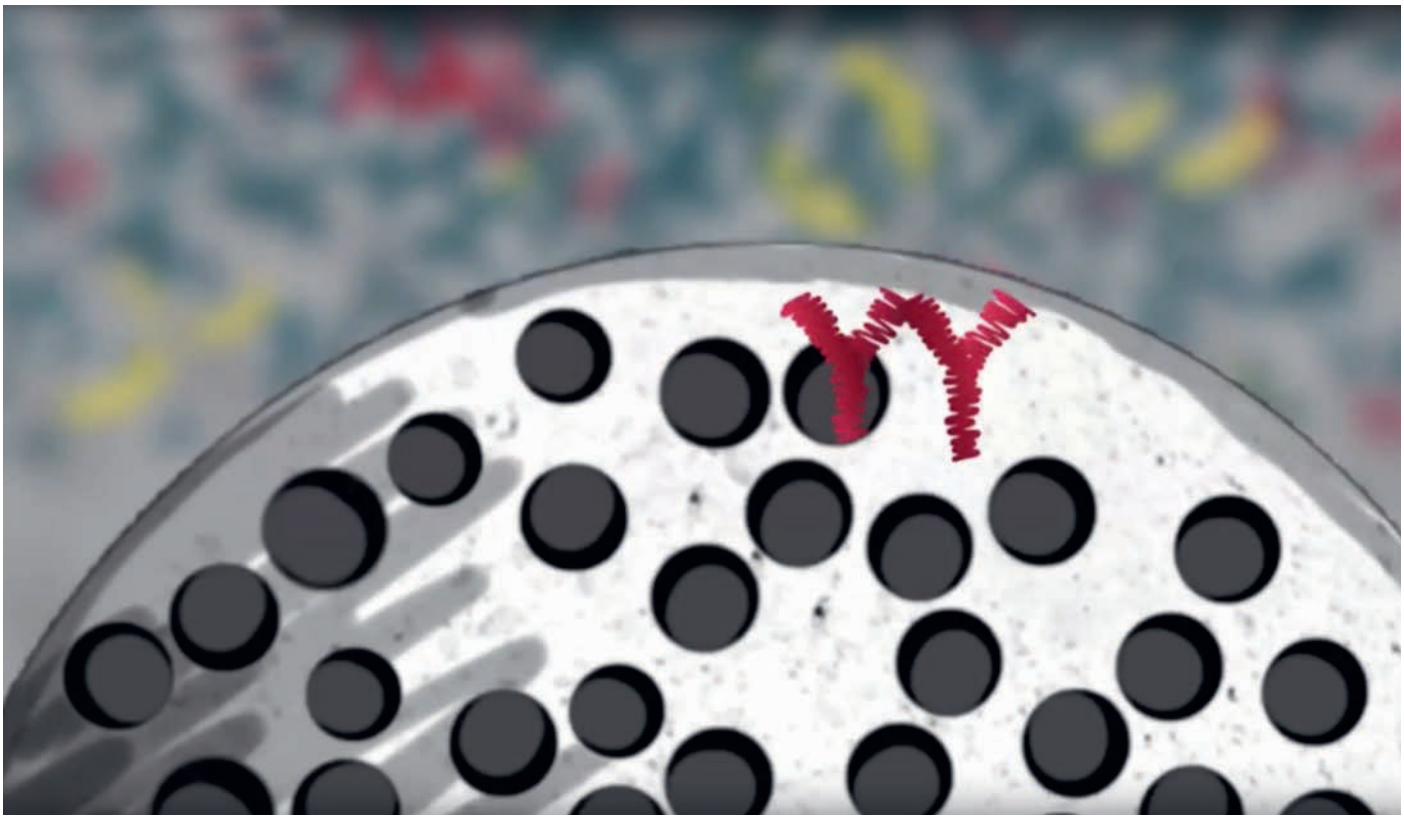
AQUEOUS SEC
GEL FILTRATION CHROMATOGRAPHY /GFC

Gel Filtration Chromatography (GFC) - SEC with aqueous mobile phase - is a popular technique for the separation of native proteins because of its non-denaturing mobile phase conditions. It enables retention of enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. Being a non-adsorptive technique SEC has limited peak capacity. For good separation, it requires that the molar mass of the molecules differ by at least twofold.

Gel filtration is typically used for the separation of proteins, monomers, aggregates and fragments, for desalting or characterization of water-soluble polymers used in food products, pharmaceutical formulations and the like.

The analysis of high and low molecular weight species of therapeutic antibody formulations is a typical GFC application in the biopharmaceutical industry.

Stationary phases for aqueous SEC range from soft packing materials, such as dextran or agarose, to hydrophilic polymers to silica. Soft particles were employed as stationary phases for early GFC whereas today porous silica particles with high mechanical strength are applied for GFC in high performance liquid chromatography (HPLC) and increasingly also in ultra high performance LC (UHPLC).



THE CHROMATOGRAPHY Y-FACTOR BY TOSOH - https://youtu.be/yAKC5b-cyvo

SEC/GFC
ABOUT TSKgel SW SERIES

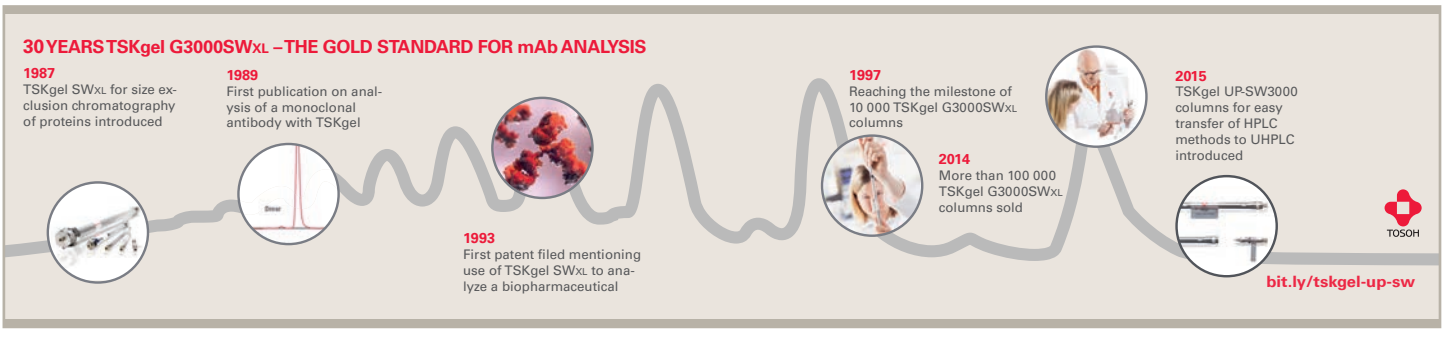
TSKgel SW series is the leading SEC column series for HPLC- and UHPLC due to its high internal pore volume, low residual adsorption and excellent resolution.

- TSKgel G3000SWXL column is the industry's gold standard for HPLC analysis of antibodies
- TSKgel UP-SW3000 columns set standards in UHPLC analysis of antibodies

TSKgel SW SERIES PROPERTIES

TSKgel SW, SWXL, SuperSW, UltraSW, and UP-SW are silica SEC phases with pore size distributions suited to protein separations. These packings feature low adsorption and well-defined pore size distribution. The 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 size exclusion columns by virtue of their large pore volumes and low residual adsorption.

Due their high resolving power, the TSKgel SW series columns are ideal for peptides, proteins and nucleic acids using an aqueous buffer as mobile phase. TSKgel G3000SWXL dominates the market of HP-SEC analysis for antibodies. TSKgel SuperSW mAb and TSKgel UP-SW3000 columns are next generation, small particle size columns for the analysis of monoclonal antibodies by HPLC and UHPLC. They 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. TSKgel SW series columns are stable from pH 2.5 to 7.5 and can be used in 100 % aqueous conditions.



SEC/GFC
TSKgel SW SERIES COLUMN SELECTION

The different pore sizes of the TSKgel SW series columns result in different exclusion limits for globular proteins as summarized in Table II. Furthermore, different particle sizes, column dimensions and housing materials are available for each of the TSKgel SW series columns. When the protein analysis needs a metal free environment, the BioAssist SW series offers TSKgel SW packings in PEEK housings.

For samples of known molecular weight, the molar mass range of the compound to be analyzed should be within the linear range of the calibration curve, representing a series of various standards with known molar masses.

For 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 G2000SWXL.

TABLE II

PROPERTIES AND SEPARATION RANGES FOR TSKgel SW TYPE PACKINGS

TSKgel column	Particle size (µm)	Pore size (nm)	Molecular weight (Da)
UP-SW2000	2	12.5	5×10^3–1.5×10^5
SuperSW2000	4	12.5	5×10^3–1.5×10^5
G2000SWXL	5	12.5	5×10^3–1.5×10^5
BioAssist G2SWXL	5	12.5	5×10^3–1.5×10^5
QC-PAK GFC 200	5	12.5	5×10^3–1.5×10^5
G2000SW	10/13	12.5	5×10^3–1.5×10^5
UP-SW3000	2	25	1×10^4–5×10^5
SuperSW3000	4	25	1×10^4–5×10^5
SuperSW mAb HTP	4	25	1×10^4–5×10^5
SuperSW mAb HR	4	25	1×10^4–5×10^5
G3000SWXL	5	25	1×10^4–5×10^5
BioAssist G3SWXL	5	25	1×10^4–5×10^5
QC-PAK GFC 300	5	25	1×10^4–5×10^5
G3000SW	10	25	1×10^4–5×10^5
UltraSW mAb Aggregate	3	30	1×10^4–2×10^6
G4000SWXL	8	45	2×10^4–7×10^6
BioAssist G4SWXL	8	45	2×10^4–7×10^6
G4000SW	13/17	45	2×10^4–7×10^6

WHICH SW COLUMN SHOULD I EVALUATE?

- Top-performer for immunoglobulin UHPLC analysis - TSKgel UP-SW3000
- First choice for immunoglobulin HPLC analysis – TSKgel SuperSW mAb series
- Analysis of smaller proteins – TSKgel UP-SW2000 or TSKgel SuperSW2000
- Analysis of larger proteins – TSKgel UltraSW Aggregate or TSKgel G4000SWXL

SEC/GFC
ABOUT TSKgel UP-SW FOR UHPLC

TSKgel UP-SW3000 columns are becoming the gold standard for mAb characterization by UHPLC by offering:

- High resolution between aggregates, monomer, and fragments
- Consistent lot-to-lot reproducibility and long column lifetime
- Ease of method transfer from HPLC to UHPLC

TSKgel UP-SW PROPERTIES

TSKgel UP-SW columns packed with 2 µm silica based particles are the latest addition to the popular TSKgel SW series. These silica-based UHPLC/HPLC columns are based on the same proven proprietary surface technology of the renowned TSKgel SW series. The surface of the particles has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups.

TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SWXL columns. Hence, methods developed using TSKgel G3000SWXL columns can easily be transferred to TSKgel UP-SW3000 columns on conventional HPLC systems as well as on UHPLC systems. Figure 2 shows the calibration curve for TSKgel UP-SW3000.

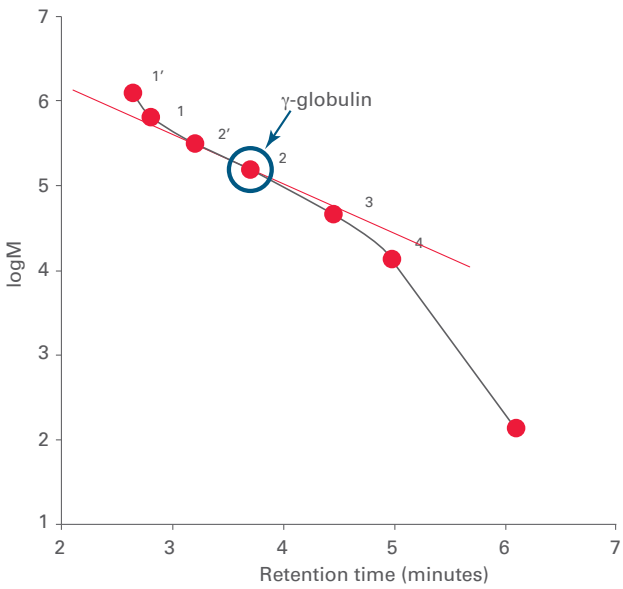
TSKgel UP-SW2000 columns feature the same pore size as TSKgel G2000SWXL columns. This is ideal for method transfer of peptide or small protein analysis from HPLC to UHPLC.

TSKgel UP-SW columns are available in 4.6 mm ID with 15 or 30 cm length. The 15 cm columns offer a shortened analysis time with improved efficiency versus the TSKgel SWXL columns. The 30 cm columns deliver dramatically increased resolution compared to the TSKgel SWXL series.

The lifetimes of TSKgel UP-SW columns are superior and can be maintained and further improved when using the corresponding guard columns. "Direct Connect" (DC) guard columns allow the user to minimize extra column dead volume.

FIGURE 2

STANDARD CALIBRATION CURVE OF QC PROTEIN STANDARD MIXTURE FOR UP-SW2000



Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm L
Mobile phase:	100 mmol/L phosphate buffer, pH 6.7 + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	1′. thyroglobulin dimer, 1. thyroglobulin, 640 kDa 2′. γ-globulin dimer , 2. γ-globulin, 155 kDa 3. ovalbumin, 47 kDa, 4. ribonuclease A, 13,700 Da 5. p-amino benzoic acid, 137 Da

SEC/GFC
ABOUT TSKgel UP-SW SERIES FOR UHPLC

REPRODUCIBILITY

TSKgel UP-SW3000 columns offer superior reproducibility injection-to-injection, from column-to-column within the same lot, and from lot-to-lot. Three consecutive injections of a protein standard mixture were analyzed, yielding low percent relative standard deviation (% RSD) for retention time for all peaks, as shown in Figure 3. A superior lot-to-lot reproducibility was proved at various biopharmaceutical labs and is the main argument for implementing this column in quality control of therapeutic antibodies.

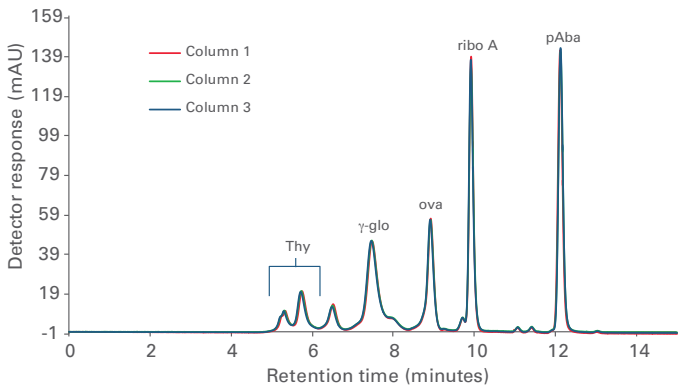
EASY METHOD TRANSFER FROM HPLC TO UHPLC

At the typical flow rate of 0.35 mL/min, the backpressure of TSKgel UP-SW3000 columns is below 30 MPa. Therefore, these columns can be used with both HPLC and UHPLC systems. However, when used with HPLC systems the benefit of reaching higher resolution due to the small particle size is not fully exploited. In order to reach resolution values similar to those achieved with UHPLC systems we recommend optimizing the HPLC system with regard to extra column dead volumes by using small I.D. capillaries and a semi-micro detector cell.

TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SWXL columns. Hence, methods developed using TSKgel G3000SWXL columns can easily be transferred to TSKgel UP-SW3000 columns. The TSKgel UP-SW3000 column offers several advantages versus the TSKgel G3000SWXL column, as shown in Figure 4 comparing the analysis of QC protein standards at the same concentrations. The TSKgel UP-SW3000 column offers higher sensitivity, with better peak shape, higher resolution and slightly shorter retention time. No change in the mobile phase composition is required; only an adjustment to a lower flow rate is necessary. A method developed on a conventional, yet optimized, HPLC system using a TSKgel UP-SW3000, 2 µm column is smoothly transferrable to a UHPLC system later.

FIGURE 3

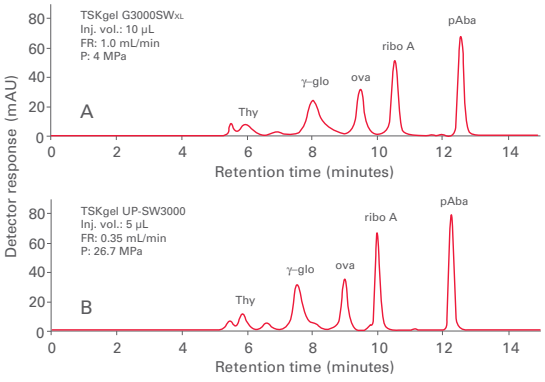
TSKgel UP-SW3000 LOT-TO-LOT REPRODUCIBILITY



Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
Instrument:	Thermo Fisher/Dionex Ultimate 3000 UHPLC System
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7 + 100mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	QC standard protein test mixture: thyroglobulin, 600 kDa, 0.5 g/L γ-globulin, 155 kDa, 1 g/L ovalbumin, 47 kDa, 1 g/L ribonuclease A, 13.7 kDa, 1.5 g/L p-aminobenzoic acid, 137 Da, 0.01 g/L

FIGURE 4

ANALYSIS OF QC PROTEIN STANDARDS



Columns:	A. TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm L B. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7 + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rates:	A. 1.0 mL/min B. 0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	A. 10 µL B. 5 µL
Samples:	1. thyroglobulin, 600 kDa 2. γ-globulin, 155 kDa 3. ovalbumin, 47 kDa 4. ribonuclease A, 13.7 kDa 5. p-amino benzoic acid, 137 Da

SEC/GFC UHPLC APPLICATIONS

SUPERIOR RESOLUTION FOR mAb ANALYSIS

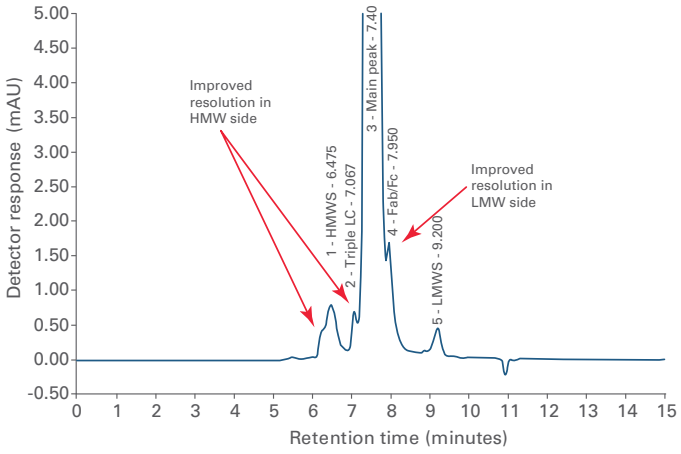
Figure 5 demonstrates the advantages of the TSKgel UP-SW3000 column for mAb analysis. TSKgel UP-SW3000 offers high resolution of both the high molecular weight (HMW) species and the Fab/Fc on the low molecular weight side. In addition, the analysis was completed in half the run time compared to a traditional 30 cm SEC column. Evaluation at customers proved that these columns are especially suitable also for the analysis of modern formats of antibody therapeutics, such as various bispecific antibodies.

FAST ANALYSIS WITH SHORT TSKgel UP-SW3000 COLUMN

Figure 6 compares the separation profile of a mAb on a 30 cm and a 15 cm length TSKgel UP-SW3000 column operated under the same mobile phase conditions and flow rate. The results indicate that the 15 cm TSKgel UP-SW3000 column provides a similar profile to the 30 cm column with 50% less run time and 50% lower backpressure at a typical flow rate of 0.35 mL/min. The resolution between dimer and monomer is still above the resolution guidelines from the USP monogram (1.2 resolution). When operated at 0.5 mL/min the same analysis can be completed in only four minutes, nearly a four times faster run time than the 30 cm length column and nearly eight times faster than a traditional SEC column.

FIGURE 5

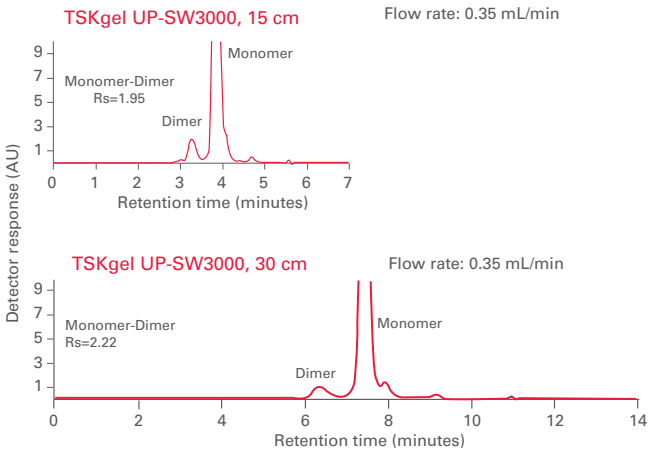
mAb ANALYSIS USING TSKgel UP-SW3000 COLUMN



Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
Instrument:	Thermo Fisher/Dionex UltiMate® 3000RS UHPLC System
Mobile phase:	0.2 mol/L potassium phosphate/0.25 mol/L KCl, pH 6.2
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	10 µL

FIGURE 6

COMPARISON OF mAb AGGREGATES ANALYSIS BETWEEN TSKgel UP-SW3000, 15 CM AND 30 CM COLUMNS



Columns:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm L TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.8, + 100 mmol/L sodium sulfate + 0.05% NaN_3
Gradient:	Isocratic
Flow rate:	as indicated in each chromatogram
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	mAb (0.4 mg/mL)

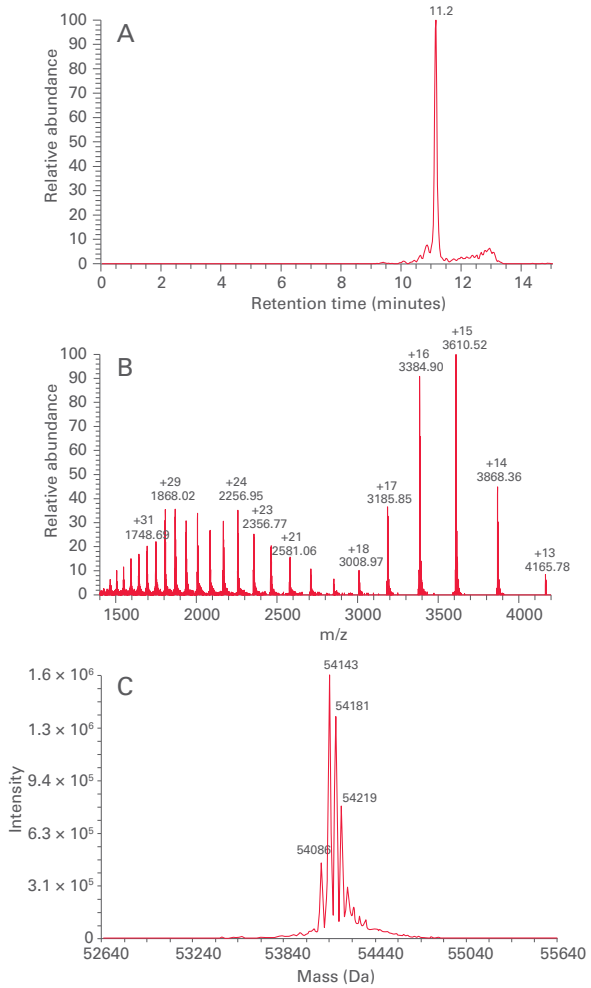
SEC/GFC UHPLC APPLICATIONS

SEC-MS ANALYSIS OF A BISPECIFIC ANTIBODY

The TSKgel UP-SW3000, 2 µm SEC column can be used for accurate molar mass determination by SEC/MS. A MS compatible mobile phase under non-denaturing condition was successfully used with the TSKgel UP-SW3000 column to analyze a Bispecific T Cell Engager (BiTE®). No signs of particle shedding or sample carryover, which may interfere with MS signal response, were noted.

FIGURE 7

SEC/MS ANALYSIS OF THE BITE



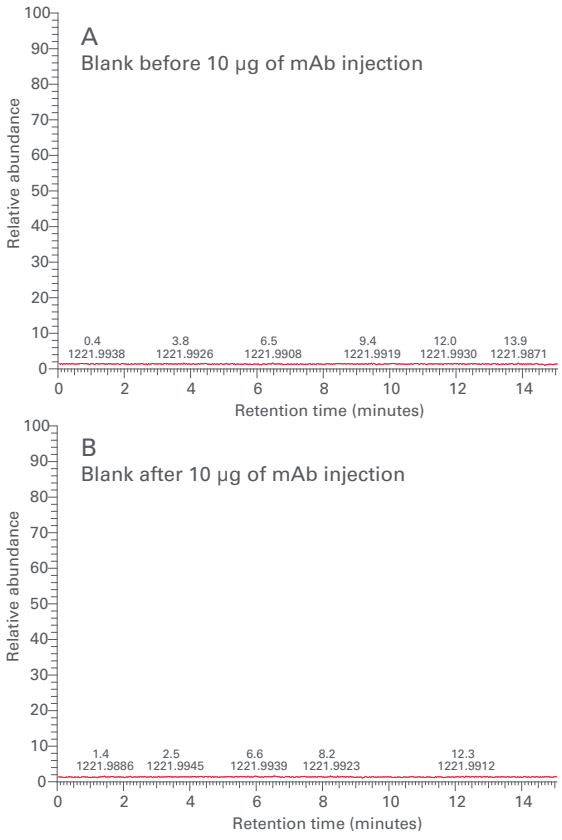
Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
HPLC Instrument:	Nexera® XR UHPLC system
MS Instrument:	Q Exactive™ Plus
Mobile phase:	20 mmol/L ammonium acetate, 10 mmol/L ammonium bicarbonate; pH 7.2
Gradient:	isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Injection vol.:	5.0 µL
Samples:	BiTE, 0.3 mg/mL (Creative Biolabs) parent mAb shown, 0.5 mg/mL (Creative Biolabs)
Ionization mode:	Electrospray ionization, positive mode
MS mode:	Scanning, m/z 800-6000

A ~55 kDa BiTE and ~150 kDa parent mAbs (data not shown) were subsequently injected onto a TSKgel UP-SW3000 column coupled to a mass spectrometer for molar mass determination. Figure 7 shows the (A) total ion chromatogram, (B) mass spectrum and (C) deconvoluted mass spectrum of the BiTE. A main peak can be seen at m/z 54,143; adjacent peaks at m/z 54,181, 54,219 and 54,086 correspond to different salt adducts.

Prior to analysis, a blank injection was run in order to assess column particle shedding. Figure 8A shows the total ion chromatogram of a blank injection that was run on a new TSKgel UP-SW3000 column. MS data indicates that there is no shedding from the column prior to sample injection. Additionally, a blank injection was run between each of the sample injections in order to monitor sample carryover. Figure 8B shows the total ion chromatogram of a blank injection run between the BiTE and parent mAb. No evidence of carryover can be seen in the run after sample injection. The lack of shedding and carryover indicates that the TSKgel UP-SW3000 column is suitable for use with MS.

FIGURE 8

COLUMN SHEDDING AND CARRYOVER ANALYSIS



SEC/GFC
ABOUT TSKgel SW mAb SERIES

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. They are compatible with HPLC and UHPLC systems.

TSKgel SW mAb PROPERTIES

TSKgel SW mAb series consists of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb):

- TSKgel SuperSW mAb HR for highest resolution over the whole range of typical mAb SEC analysis, from fragments to aggregates.
- TSKgel SuperSW mAb HTP features the same stationary phase as the HR column but has smaller column dimensions for high throughput mAb analysis
- TSKgel UltraSW Aggregate was developed to offer a wider separation range in the molecular mass range of antibody aggregates and high molecular weight proteins

Compared to competitive columns, these stainless steel, silica-based TSKgel columns offer reduced lot-to-lot variation, longer column life, reduction of unspecified adsorption, and superior recovery of aggregates.

The HR designation represents the high resolution analysis, while the HTP stands for "high throughput" due to the shorter analysis time. The TSKgel UltraSW Aggregate phase provides smaller particle size and a higher exclusion limit through slightly larger pores.

Table III shows a summary of the product attributes for the TSKgel SW mAb columns. These columns utilize a unique pore-controlled technology, which produces a shallow calibration curve in the molar mass region of a typical monoclonal antibody. As shown in Figure 9, the calibration curve for the TSKgel SuperSW mAb HR column is similar to that of the TSKgel G3000SWXL column curve and has a shallower slope than the TSKgel UltraSW Aggregate column around the molar mass range of gamma-globulin. This shallow calibration curve produces high resolution separations. The TSKgel UltraSW Aggregrate calibration curve shows a separation range up to around 2 million Da, which implies better resolution of aggregate/multimer of a mAb.

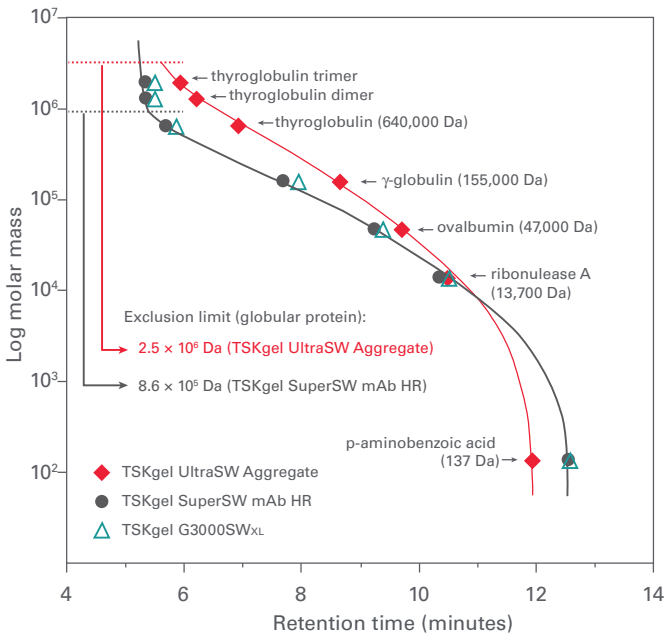
TABLE III

PRODUCT ATTRIBUTES

TSKgel column	SuperSW mAb HR	SuperSW mAb HTP	UltraSW Aggregate
Base material		Silica	
Particle size (mean)	4 µm	4 µm	3 µm
Pore size (mean)	25 nm	25 nm	30 nm
Functional group		Diol	
pH stability		2.5-7.5	
Calibration range	1×10^4 - 5×10^5 Da (globular proteins)	1×10^4 - 5×10^5 Da (globular proteins)	1×10^4 - 2×10^6 Da (globular proteins)

FIGURE 9

PROTEIN CALIBRATION CURVES FOR TSKgel SW mAb COLUMNS



SEC/GPC ANTIBODY APPLICATIONS

FAST ANALYSIS OF mAb AGGREGATION

The shorter column length allows the TSKgel SuperSW mAb HTP column to provide fast and efficient run times in the separation of a mAb monomer and dimer. Compared with a conventional mAb analysis on a 30 cm length column, analysis time can be cut to half without compromising resolution too much. Figure 10 shows the optimization of mAb aggregate analysis on TSKgel SuperSW mAb HTP with regard to analysis time. Resolution is still high enough for quantitation.

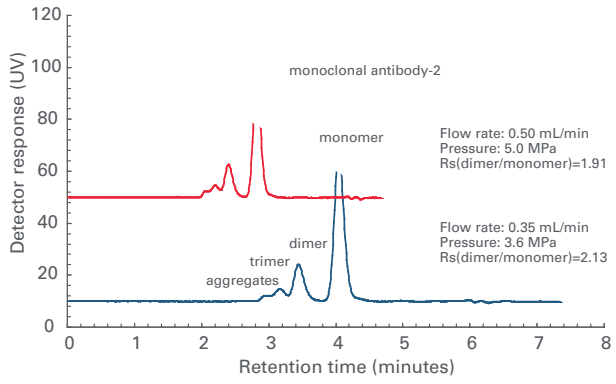
ANALYSIS OF mAb FRAGMENTS

Recent research has shown an interest in mAb half-bodies as therapeutic vectors as they can be further targeted for conjugation, enzyme labeling, or antibody immobilization. Mab half-bodies can be generated through genetic engineering or by selective reduction of hinge-region disulfide bonds present in the mAb by mild reducing agents, such as TCEP [tris(2carboxyethyl) phosphine]. A mAb half-body was generated through protein reduction using TCEP and subsequently identified by gel electrophoresis.

Figure 11 illustrates the separation of human IgG monomer, half-body (70 kDa) and fragment (1/3 mAb) using a TSKgel SuperSW mAb HR column. High resolution (Rs = 1.13) of the IgG monomer and half-body species was achieved.

FIGURE 10

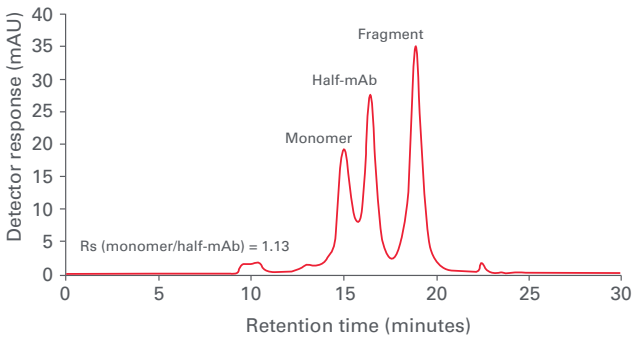
HIGH SPEED SEPARATION OF THERAPEUTIC mAb



Column:	TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm L
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 11

SEPARATION OF HUMAN IgG MONOMER, HALF-BODY, AND FRAGMENTS USING A TSKgel SuperSW mAb HR COLUMN



Column:	TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm L
Mobile phase:	0.1 mol/L phosphate/0.1 mol/L sulfate buffer + 0.05% NaN_3
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	human IgG (4.6 g/L) from Sigma Aldrich

SEC/GFC
ANTIBODY AGGREGATE APPLICATIONS

SEPARATION OF HIGHER ANTIBODY AGGREGATES

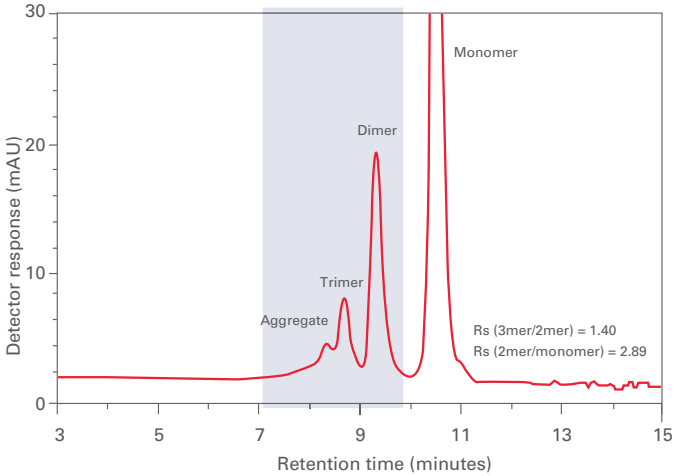
TSKgel UltraSW Aggregate has a smaller particle size than the SuperSW material, and offers high resolution separation of mAb multimers. Figure 12 shows the analysis of a mouse-human chimeric IgG using the TSKgel UltraSW Aggregate column. Superior resolution of the mAb trimer and dimer is obtained. The 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.

ANALYSIS OF A LARGE METALLOPROTEIN

TSKgel UltraSW Aggregate provides a larger pore size than TSKgel mAb HR. 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 13. 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 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 12

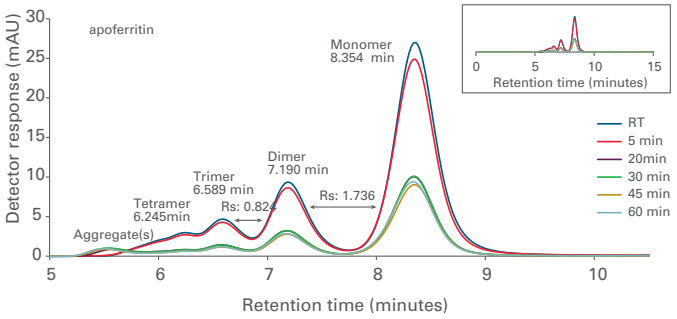
SEPARATION OF mAb TRIMER AND DIMER



Column:	TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm L
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 13

ANALYSIS OF FORCED DENATURED APOFERRITIN



Protein	Molecular weight (kDa)			
	Monomer	Dimer	Trimer	Tetramer
ferritin and apoferritin	450	900	1.350	1.800

Column:	TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm L
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

SEC/GFC
ABOUT TSKgel SW, SWXL, SuperSW

TSKgel SW, SWXL and SuperSW stationary phases are all based on spherical silica particles with very high internal pore volumes. They are available in various particle and pore sizes. All SW-type columns feature low residual adsorption, essential for gel filtration.

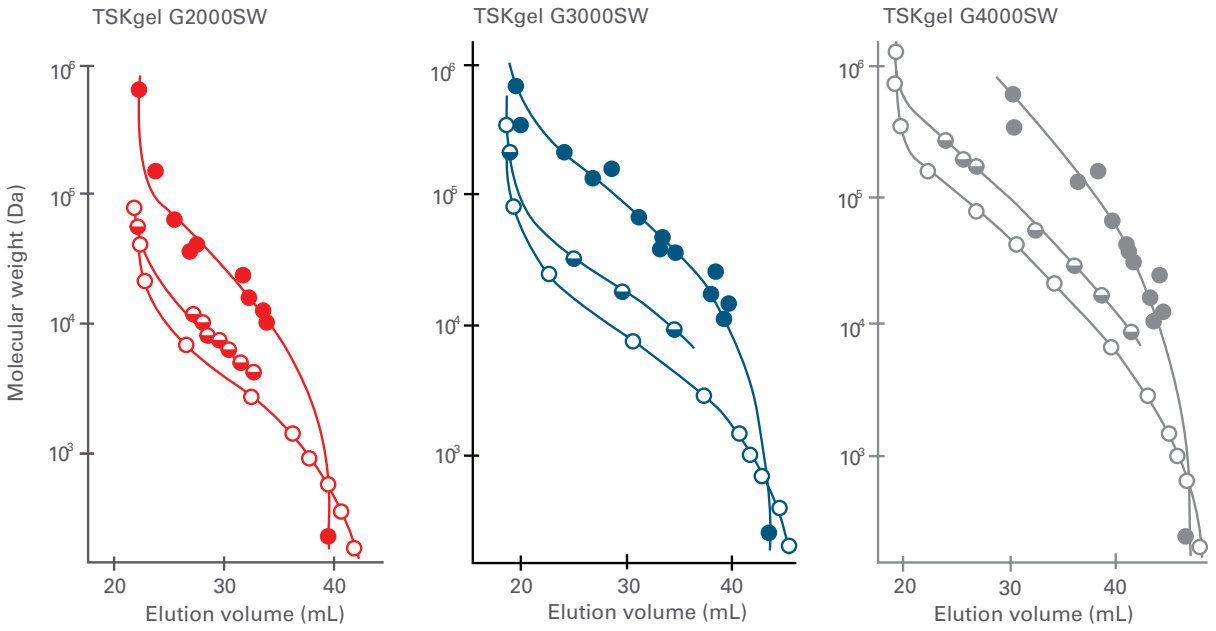
TSKgel SW SERIES PROPERTIES

TSKgel SW columns, introduced in 1977, were the first of a long line of high performance gel filtration columns that have become synonymous with isolating proteins and analyzing protein molar masses for biotechnology applications. Particles having three different pore sizes are available TSKgel G2000SW (12.5 nm pores), TSKgel G3000SW (25 nm pores), and TSKgel G4000SW (45 nm pores).

The TSKgel G2000SW column provides excellent separation of peptides and proteins with molar masses up to 1.0×10^5 Da. TSKgel G3000SW columns are the best choice for separation of proteins and other biomolecules with molar masses up to 5.0×10^5 Da (e.g. IgG), while TSKgel G4000SW columns are preferred for proteins and other biomolecules of even higher molar masses. Figure 14 shows the calibration curve for globular proteins, polyethylene oxides, and dextrans for each of the three TSKgel SW columns.

FIGURE 14

POLYETHYLENE OXIDE, DEXTRAN AND PROTEIN CALIBRATION CURVES FOR TSKgel SW COLUMNS



Column: TSKgel SW, two 7.5 mm ID × 60 cm L columns in series
Mobile phase: 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
Sample: ● proteins, ○ polyethylene oxides, ◒ dextrans

SEC/GFC
ABOUT TSKgel SWXL

TSKgel SWXL SERIES PROPERTIES

TSKgel SWXL columns, introduced in 1987, are packed with 5 µm or 8 µm particles to improve sample resolution or to reduce analysis time over TSKgel SW columns. They are available in the same grades as TSKgel SW columns G2000SWXL (12.5 nm), G3000SWXL (25 nm), and G4000SWXL (45 nm).

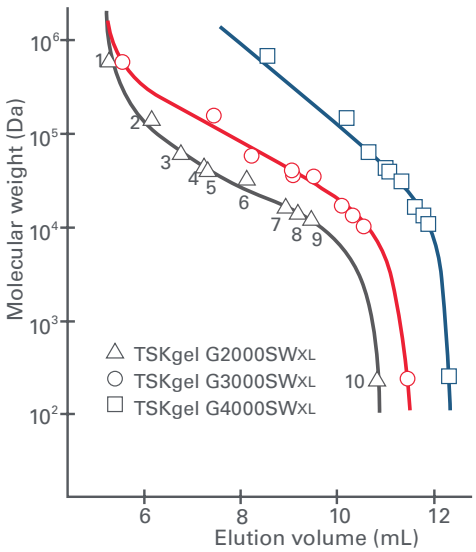
Many different hardware formats are available within the TSKgel SWXL line. TSKgel BioAssist columns are made of PEEK housing material to reduce sample adsorption to stainless steel or glass. Also available within the TSKgel G2000SWXL and G3000SWXL line are QC-PAK columns. These columns are 15 cm in length with 5 µm particles and offer the same resolution in half the time as the 30 cm, 10 µm TSKgel G2000SW and G3000SW columns.

COLUMN SELECTION

TSKgel SWXL columns are commonly used in the quality control of monoclonal antibodies and other biopharmaceutical products. TSKgel G2000SWXL columns are an excellent choice for small proteins and peptide separations. proteins and antibodies are separated well on TSKgel 3000SWXL columns, while TSKgel G4000SWXL provides the largest exclusion limit and the widest fractionation range. It is an excellent choice for pegylated proteins or glycosylated biomolecules. Figure 15 shows the calibration curves for globular proteins for each of the three TSKgel SWXL columns.

FIGURE 15

CALIBRATION CURVES FOR TSKgel SWXL COLUMNS



Columns:	A. TSKgel SWXL, 5 or 8 µm, 7.8 mm ID × 30 cm L
Mobile phase:	0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection:	UV @ 220 nm
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)

SEC/GFC
TSKgel SWXL APPLICATIONS

SEC-MALS ANALYSIS OF ANTIBODY AGGREGATION

G3000SWXL is the industry standard for aggregation analysis in quality control of monoclonal antibodies. Figure 16 depicts the analysis of mAb Aggregates with UV, refractive index (RI) and multi angle light scattering (MALS) detection.

SIZE VARIANT ANALYSIS OF CONJUGATES

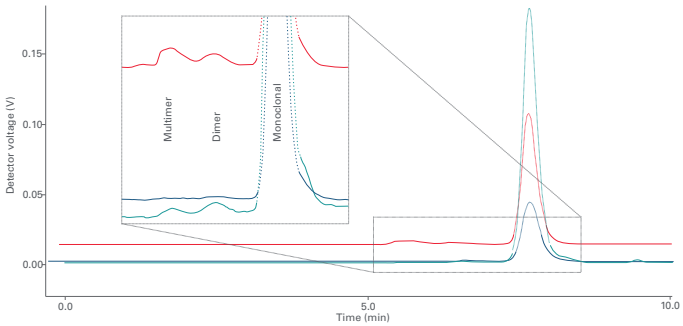
A sample of both conjugated (T-DM1) and unconjugated (Trastuzumab) monoclonal antibody was analyzed on a TSKgel G3000SWXL column with a phosphate-buffered saline mobile phase. The use of this inorganic mobile phase for the unconjugated mAb showed no the expected results.

For the analysis of the conjugated mAb (ADC) in the inorganic mobile phase, poor peak shape (greatly increased tailing) and incomplete resolution of aggregates from the monomeric conjugated antibody were observed (see A in Figure 17).

The addition of an organic modifier to the mobile phase, in this case 15% 2-propanol, restored peak shape and resolution of the conjugated mAb analyzed on a TSKgel G3000SWXL column (B in Figure 17). These results indicate that the attached hydrophobic drugs lead to non-specific interaction between the ADC and the column stationary phase. The addition of organic solvents to the mobile phase can be used to overcome non-specific interactions between the ADC and the column stationary phase.

FIGURE 16

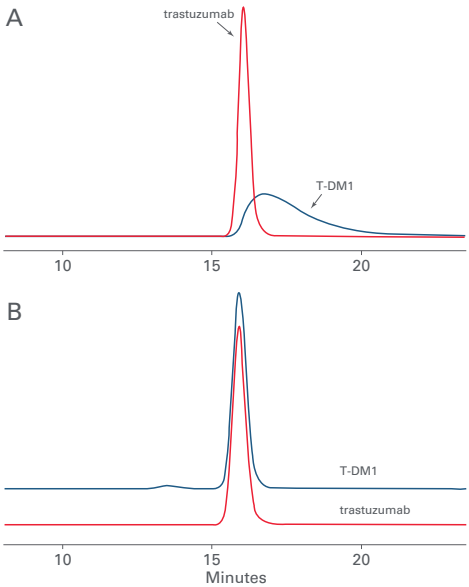
SEC-MALS-UV-RI ANALYSIS OF mAb AGGREGATES



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

FIGURE 17

SIZE VARIANT ANALYSIS OF CONJUGATES



Column: TSKgel G3000SWXL, 7.8 mm ID × 30 cm L
Mobile phase: A: 0.2 mol/L KPi and 0.25 mol/L KCl, pH 6.95
B: 85% KPi/KCl + 15% 2-propanol
Flow rate: 0.5 mL/min
Detection: UV @ 280 nm

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SEC/GFC
TSKgel SWXL APPLICATIONS

CHARACTERIZATION STUDIES OF PEGYLATED LYSOZYME

Chemical modification of therapeutic proteins in order to enhance their biological activity is of increasing interest.

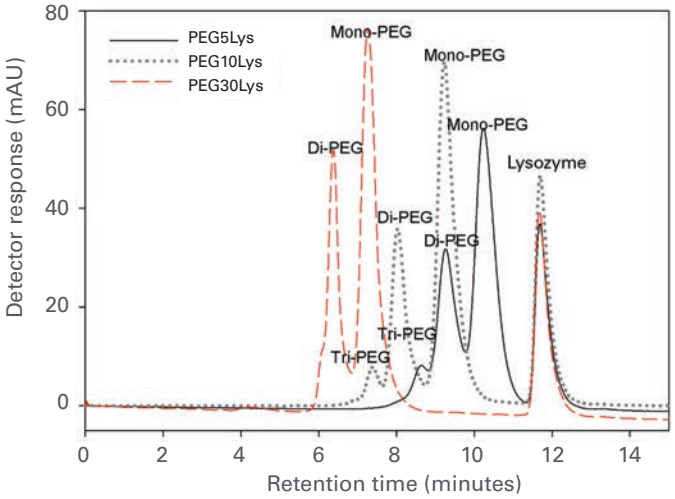
One of the most frequently used protein modification methods, PEGylation, changes the biochemical and physicochemical properties of the protein, which can result in several important benefits, among them more effective target delivery, slower in vivo clearance, and reduced toxicity and immunogenicity 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 18. 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 was observed by size exclusion chromatography.

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 18

SEC ANALYSIS OF PEGYLATION REACTION MIXTURES



Column:	TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm L
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/GFC
ABOUT TSKgel SuperSW

TSKgel SuperSW SERIES PROPERTIES

TSKgel SuperSW columns, introduced in 1997, contain smaller particles than TSKgel SWXL columns; 4 µm versus 5 µm. In addition, the column internal diameter has been reduced from 7.8 mm ID to 4.6 mm ID to provide higher sensitivity in sample-limited cases and to cut down on solvent use.

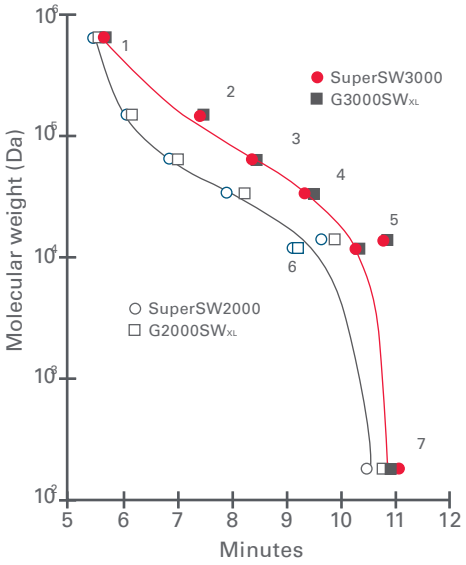
It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on these columns. See page 29 (Size Exclusion Tips) for recommendations on minimizing the dead volume in the HPLC system.

The following phases are available within the TSKgel SuperSW column line: TSKgel SuperSW2000 (12.5 nm pores) and TSKgel SuperSW3000 (25 nm pores):

The 12.5 nm pore size of the TSKgel SuperSW2000 columns results in a fractionation range up to 1.5 × 10^5 Da for globular proteins, ideally suited for peptides and small proteins. TSKgel SuperSW3000 columns have a fractionation range up to 5.0 × 10^5 Da for globular proteins, the perfect range for immunoglobulins. Since both columns have a 4.6 mm inner diameter, they are ideal for sample-limited applications. TSKgel SuperSW3000 is even available in 2.0 and 1.0 mm ID, when sample amount is even more limited. Figure 19 shows the calibration curves for TSKgel SuperSW columns compared to TSKgel SWXL.

FIGURE 19

CALIBRATION CURVES FOR TSKgel SWXL AND SuperSW



Columns:	TSKgel SWXL, 5 µm, 7.8 mm ID x 30 cm L TSKgel SuperSW, 4 µm, 4.6 mm ID x 30 cm L
Mobile phase:	0.15 mol/L phosphate buffer (pH 6.8)
Flow rate:	0.35 mL/min for SuperSW; 1.0 mL/min for SWXL
Temperature:	25 °C
Detection:	UV @ 280 nm (220 nm for triglycine)
Sample:	proteins: 1. thyroglobulin (660,000 Da); 2. γ-globulin (150,000 Da); 3. BSA (67,000 Da); 4. b-lactoglobulin (18,400 Da); 5. lysozyme (14,500 Da); 6. cytochrome C (12,400 Da); 7. triglycine (189 Da)

SEC/GFC
TSKgel SuperSW APPLICATIONS

TRACE LEVELS OF PROTEINS

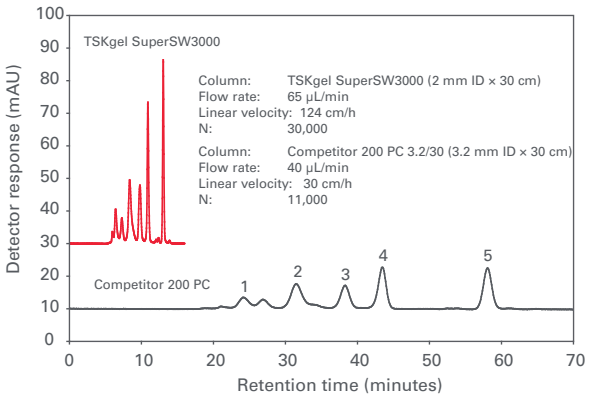
Figure 20 shows a comparative separation of several standard proteins at low level concentrations on a 2 mm ID TSKgel SuperSW3000 column and on a competitive GFC column. The TSKgel SuperSW3000 column is an excellent choice for the rapid analysis of proteins at trace levels, showing improved peak shape and superior resolution.

SEPARATION OF PEPTIDES AND PROTEINS

Figure 21 shows an example of a mixture of peptides and small proteins separated on TSKgel SuperSW2000. The analysis of insulin and insulin aggregates in the biopharmaceutical industry is a typical application for TSKgel SuperSW2000.

FIGURE 20

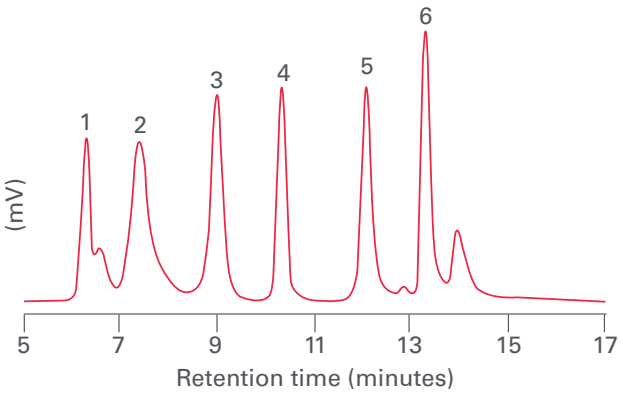
ANALYSIS OF STANDARD PROTEINS AT LOW CONCENTRATIONS



Columns:	TSKgel SuperSW3000, 4 µm, 2 mm ID × 30 cm L
	Competitor 200 PC 3.2/30, 13 µm, 3.2 mm ID × 30 cm L
Mobile phase:	0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4
	+ 0.05% NaN_3, pH 6.7
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	0.2 µL
Samples:	1. thyroglobulin (1.0 g/L)
	2. β-globulin (2.0 g/L)
	3. ovalbumin (2.0 g/L)
	4. ribonuclease A (3.0 g/L)
	5. p-aminobenzoic acid (0.02 g/L)

FIGURE 21

SEPARATION OF PROTEINS AND PEPTIDES



Column:	TSKgel SuperSW2000, 4 µm, 4.6 mm ID × 30 cm L
Mobile phase:	0.2 mol/L phosphate buffer (pH 6.7)
Flow rate:	0.35 mL/min
Detection:	UV/VIS @ 220 nm (micro-cell)
Injection vol.:	5 µL
Samples:	1) thyroglobulin
	2) γ-globulin
	3) ovalbumin
	4) myoglobin
	5) insulin
	6) oxytocin
Sample Load:	0.1 g/L

SEC/GFC
SEC TIPS

TSKgel size exclusion columns are offered in glass, PEEK (polyetheretherketone), and stainless steel (SS) hardware. SS or Pyrex® frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings.

Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that are exposed to the mobile phase and sample.

Good laboratory procedures demand that the analytical column be protected by a guard column. Packed guard columns are available for use with TSKgel size exclusion columns.

TSKgel size exclusion columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, an OCS Sheet summarizing the recommended operating conditions for optimum column performance and a general TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems.

When using TSKgel SuperSW, Ultra SW or UP-SW columns it is important to employ an HPLC system that is optimized with regards to extra-column volume to take full advantage of the high column efficiency that can be obtained on these columns.

Components such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant may require optimization:

For best results, it is recommended to use the following conditions for TSKgel SuperSW, UltraSW, and UP-SW

- Suppress peak broadening by reducing extra-column volume in connecting tubing between injector, guard column, analytical column, and detector. Use 0.004” or 0.005” ID (0.100 mm or 0.125 mm) tubing, when available, and as short a length as is practical.
- When working with a UV detector, install a micro flow cell or a low dead volume-type cell. Low dead volume type cells are effective in high-sensitivity analysis.
  (Use of a standard cell is also possible. However, theoretical plates will be approximately 80% of those obtained with a micro flow cell.)
- Prevent the sample volume from causing extra-column band broadening due to volume overloading. You can test this by injecting half the sample volume and measuring peak efficiency. Sample injection volume should be 1-10 µL. Sample load should be 100 µg or smaller. A low dispersion injector is recommended.
- The pump(s) should work reproducible at low flow rates, as the recommended flow rate range is 0.1-0.35 mL/min.
- We recommend that you install a guard column or at least a guard filter to protect your TSKgel column.

SEC/GFC
ORDERING INFORMATION TSKgel SW SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel UP-SW UHPLC Columns						
0023514	UP-SW2000	4.6	30	2	≥ 45,000	34.0
0023515	UP-SW2000	4.6	15	2	≥ 25,000	25.0
0023448	UP-SW3000	4.6	30	2	≥ 45,000	34.0
0023449	UP-SW3000	4.6	15	2	≥ 25,000	25.0
TSKgel SW mAb Columns						
0022854	SuperSW mAb HR	7.8	30	4	≥ 30,000	12.0
0022855	SuperSW mAb HTP	4.6	15	4	≥ 15,000	8.0
0022856	UltraSW Aggregate	7.8	30	3	≥ 35,000	12.0
TSKgel SW Standard Columns						
0018674	SuperSW2000	4.6	30	4	≥ 30,000	12.0
0008540	G2000SWXL	7.8	30	5	≥ 20,000	7.0
0016215	QC-PAK GFC 200	7.8	15	5	≥ 10,000	4.0
0005788	G2000SW	7.5	30	10	≥ 10,000	2.0
0005102	G2000SW	7.5	60	10	≥ 20,000	4.0
0006727	G2000SW	21.5	30	13	≥ 10,000	1.0
0005146	G2000SW	21.5	60	13	≥ 20,000	2.0
0021845	SuperSW3000	1.0	30	4	≥ 18,000	12.0
0021485	SuperSW3000	2.0	30	4	≥ 25,000	12.0
0018675	SuperSW3000	4.6	30	4	≥ 30,000	12.0
0008541	G3000SWXL	7.8	30	5	≥ 20,000	7.0
0016049	QC-PAK GFC 300	7.8	15	5	≥ 10,000	4.0
0005789	G3000SW	7.5	30	10	≥ 10,000	2.5
0005103	G3000SW	7.5	60	10	≥ 20,000	5.0
0006728	G3000SW	21.5	30	13	≥ 10,000	1.5
0005147	G3000SW	21.5	60	13	≥ 20,000	3.0
0008542	G4000SWXL	7.8	30	8	≥ 16,000	3.5
0005790	G4000SW	7.5	30	13	≥ 8,000	1.5
0005104	G4000SW	7.5	60	13	≥ 16,000	3.0
0006729	G4000SW	21.5	30	17	≥ 8,000	1.0
0005148	G4000SW	21.5	60	17	≥ 16,000	2.0
TSKgel SW Glass Columns						
0008800	G3000SW, Glass	8.0	30	10	≥ 10,000	2.0
0008801	G4000SW, Glass	8.0	30	13	≥ 8,000	2.0
TSKgel SW PEEK Columns						
0020027	BioAssist G2SWXL	7.8	30	5	≥ 20,000	7.0
0020026	BioAssist G3SWXL	7.8	30	5	≥ 20,000	7.0
0020025	BioAssist G4SWXL	7.8	30	8	≥ 16,000	3.5

SEC/GFC
ORDERING INFORMATION TSKgel SW SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	
Guardcolums					
0023516	UP-SW2000 Guardcolumn	4.6	2.0	2	For all UP-SW2000
0023517	UP-SW2000 Guardcolumn DC	4.6	2.0	2	For all UP-SW2000
0023450	UP-SW3000 Guardcolumn	4.6	2.0	2	For all UP-SW3000
0023451	UP-SW3000 Guardcolumn DC	4.6	2.0	2	For all UP-SW3000
0022857	SuperSW mAb Guardcolumn	6.0	4.0	4	For SuperSW mAb HR
0022858	SuperSW mAb Guardcolumn	3.0	4.0	4	For SuperSW mAb HTP
0022859	UltraSW Guardcolumn	6.0	4.0	3	For all UltraSW Aggregate
0018762	SuperSW Guardcolumn	4.6	3.5	4	For 4.6 mm ID SuperSW columns
0008543	SWXL Guardcolumn	6.0	4.0	7	For all SWXL columns and P/Ns 0016215 and 0016049 (contains 3000SWXL packing)
0005371	SW Guardcolumn	7.5	7.5	10	For all 7.5 mm ID SW columns (contains 3000SW packing)
0005758	SW Guardcolumn	21.5	7.5	13	For all 21.5 mm ID SW columns
0018008	BioAssist SWXL Guardcolumn	6.0	4.0	7	For all BioAssist SWXL, PEEK columns
Glass Guardcolums					
0008805	SW Guardcolumn, Glass	8.0	4.0	10	For all 8 mm ID SW glass columns
Bulk packing					
0006819	SW Top-Off, 1g wet gel			10	For all 7.5 mm ID SW columns
0008544	SWXL Top-Off, 1g wet gel			5	For SWXL and QC-PAK columns

SEC/GFC
ABOUT TSKgel PW SERIES

TSKgel PW series phases are hydrophilic, rigid, spherical, porous methacrylate beads for aqueous SEC:

- pH range of 2 to 12, with up to 50% polar organic solvent
- Wide choice of pore sizes for separation ranges up to 2 x 10^7 Da for linear polymers
- Linear SEC column line incorporating proprietary multi-pore technology

TSKgel PW SERIES PROPERTIES

TSKgel PW and TSKgel PWXL polymer based stationary phases 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.

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. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in Table IV.

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 with linear calibration curves for MW distribution analysis.

RECOMMENDED MOBILE PHASES

TSKgel PW series columns are stable in broad pH range from pH 2 to 12 and can be used in aqueous mobile phases. 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.

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.

TSKgel PW phases 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 water-soluble, organic modifier such as acetonitrile. All TSKgel PW series packings are compatible with 20% aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethylformamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50% aqueous acetone.

Typical examples of mobile phases for a variety of sample types are given in Table V.

SEC/GFC
TSKgel PW SERIES COLUMN SELECTION

TABLE IV

PROPERTIES AND SEPARATION RANGES FOR TSKgel PW-TYPE PACKINGS

TSKgel Column	Particle size (µm)	Pore size (nm)	MW range		
			(PEG/PEO)	Dextrans*	Globular Proteins
G2000PW	12	12.5	$< 2 \times 10^3$	-	$< 5 \times 10^3$
G2500PW	12, 17	< 20	$< 3 \times 10^3$	$< 3 \times 10^3$	$< 8 \times 10^3$
G3000PW	12, 17	20	$< 5 \times 10^4$	$< 6 \times 10^4$	5×10^2 - 8×10^5
G4000PW	17	50	$< 3 \times 10^5$	1×10^3 - 7×10^5	1×10^4 - 1.5×10^6
G5000PW	17	100	$< 1 \times 10^6$	5×10^4 - 2.5×10^6	$< 1 \times 10^8$
G6000PW/ BioAssist G6PW	17	> 100	$< 8 \times 10^6$	5×10^5 - 5×10^7	$< 2 \times 10^8$
GMPW	17	< 10 - 100	5×10^2 - 8×10^6	$< 5 \times 10^7$	$< 2 \times 10^8$
G2500PW$_{XL}$	7	< 20		$< 3 \times 10^3$	$< 8 \times 10^3$
G3000PW$_{XL}$	7	20	$< 5 \times 10^4$	$< 6 \times 10^4$	5×10^2 - 8×10^5
G4000PW$_{XL}$	10	< 50	$< 3 \times 10^5$	1×10^3 - 7×10^5	1×10^4 - 1.5×10^6
G5000PW$_{XL}$	10	100	$< 1 \times 10^6$	5×10^4 - 2.5×10^6	$< 1 \times 10^8$
G6000PW$_{XL}$	13	> 100	$< 8 \times 10^6$	5×10^5 - 5×10^7	$< 2 \times 10^8$
G-DNA-PW	10	> 100	$< 8 \times 10^6$	$< 5 \times 10^7$	
GMPW$_{XL}$	13	10 - 100	5×10^2 - 8×10^6	$< 5 \times 10^7$	$< 2 \times 10^8$
G-Oligo-PW	7	12.5	$< 3 \times 10^3$		$< 5 \times 10^3$
SuperMultiporePW-N	4	n/a	3×10^2 - 5×10^4		
SuperMultiporePW-M	5	n/a	5×10^2 - 1×10^6		
SuperMultiporePW-H	8 (6-10)	n/a	1×10^3 - 1×10^7		
SuperOligoPW	3	n/a	1×10^2 - 3×10^3		
G3000PW$_{XL}$-CP	7	20	$< 9 \times 10^4$		
G5000PW$_{XL}$-CP	10	100	$< 1 \times 10^6$		
G6000PW$_{XL}$-CP	13	> 100	$< 2 \times 10^7$		

Column: TSKgel PW columns, 7.5 mm ID x 60 cm L; TSKgel PW$_{XL}$, TSKgel PW$_{XL}$-CP, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L
Mobile phase: 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

SEC/GFC
TSKgel PW MOBILE PHASE SELECTION

TABLE V

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 0.01 mol/L 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)

SEC/GFC
ABOUT TSKgel PW/PWXL

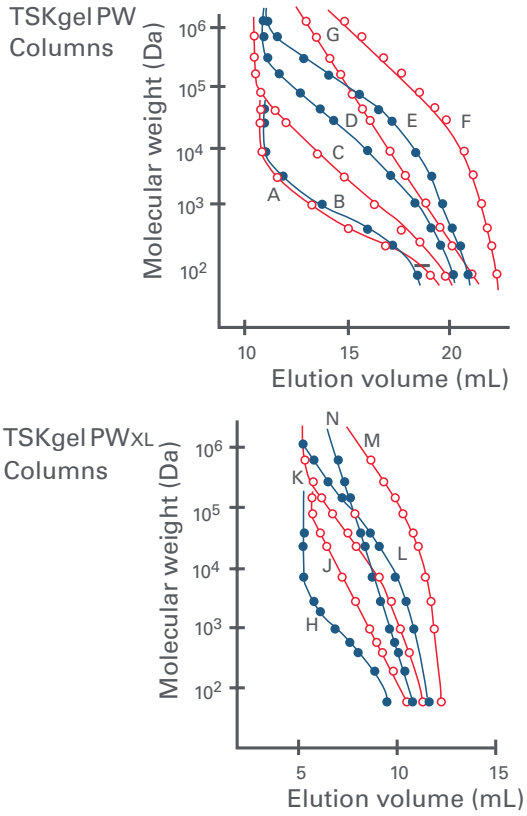
TSKgel PW AND PWXL SERIES PROPERTIES

TSKgel PW and TSKgel PWXL columns are available for a broad range of molecular weights. Mixed bed columns (TSKgel GMPW/GMPWXL) allow the analysis of a broad range of polymers in one run.

TSKgel PW and TSKgel PWXL columns are commonly used for the separation of synthetic polymers, oligosaccharides, nucleic acids, small viruses or virus like proteins. They can be also used for protein separations if solvent pH or mass range exceeds that of silica based SEC column, such as TSKgel SW series. Compared to TSKgel PW series, TSKgel SW shows better resolution and peak shapes for protein separation.

FIGURE 22

POLYETHYLENE GLYCOL AND OXIDE CALIBRATION CURVES ON TSKgel PW AND TSKgel PWXL COLUMNS



Column: TSKgel PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm ID x 60 cm L
TSKgel PWXL columns: H. G2500PWXL, J. G3000PWXL, K. G4000PWXL, L. G5000PWXL, M. G6000PWXL, N. GMPWXL, all 7.8 mm ID x 30 cm L
Elution: distilled water
Flow rate: 1.0 m L/min
Detection: RI

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers mixed-bed and multi-pore columns for analysis. The mixed bed column TSKgel GMPW and its high resolution counterpart, TSKgel GMPWXL, are packed with the G2500PW, G3000PW and G6000PW or corresponding PWXL resins.

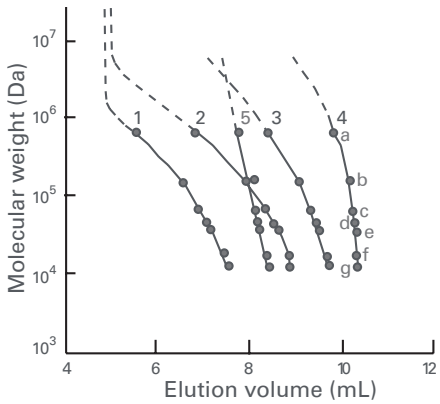
They offer a broad molecular weight separation range. As shown below, the calibration curve for polyethylene glycols and oxides on these columns is linear over the range of 100-1,000,000 Da.

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.

The multi-pore columns offering near-linear calibration curves are described in detail on page 37.

FIGURE 23

PROTEIN CALIBRATION CURVES ON TSKgel PWXL COLUMNS



Column: 1. G3000PWXL, 2. G4000PWXL, 3. G5000PWXL, 4. G6000PWXL, 5. GMPWXL
Mobile phase: 0.2 mol/L phosphate buffer (pH 6.8)
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Sample: a. thyroglobulin (660,000 Da), b. γ-globulin (150,000 Da), c. albumin (67,000 Da), d. ovalbumin (43,000 Da), e. b-lactoglobulin (36,000 Da), f. myoglobin (16,900 Da), g. cytochrome C (12,400 Da)

SEC/GFC
TSKgel PW AND PWXL APPLICATIONS

COLUMN SELECTION

For samples of known molecular weight, the molar mass range of the compound to be analyzed should be within the linear range of the calibration curve, representing a series of various standards with known molar masses. Figure 22 shows the calibration curves for polythylene glycols (PEG) and polyethylene oxides (PEO) on TSKgel PW and PWXL columns. Figure 23 shows protein calibration curves on TSKgel PWXL columns.

OLIGOSACCHARIDES

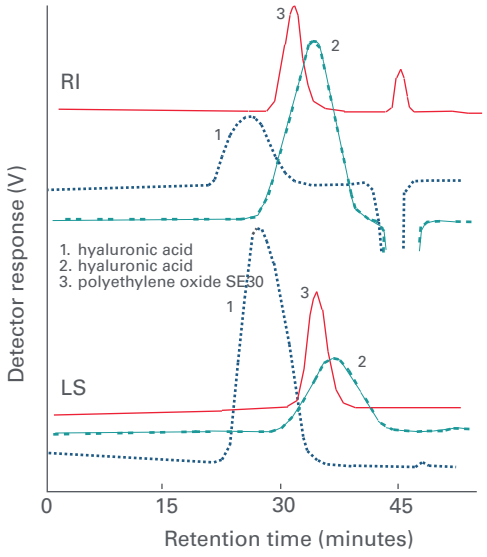
TSKgel PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molar mass distribution. An effective separation of the anionic hydrophilic glucosaminoglycan, hyaluronic acid, is shown in Figure 24 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase. Detection was performed with refractive index) RI) and light scattering (LS). To obtain shorter analysis time and similar resolution, we recommend using TSKgel G3000PWXL and G4000PWXL columns in series.

QUANTIFICATION AND CHARACTERIZATION OF VIRUS-LIKE PARTICLES

TSKgel PWXL material is well suited to quantify and characterize virus like particles (VLPs) by SEC-UV or SEC-MALS. Figure 25 shows how a TSKgel G5000PWXL column was applied to quantify and characterize HIV-1 gag VLPs. A 25 mM Na-phosphate, 250 mM NaCl, pH 8.0 buffer at a flow rate of 0.3 mL/min was used for calibration. The HIV-1 gag VLP standard material or samples were optionally diluted in the elution buffer. (Data kindly provided by Petra Steppert, University of Natural Resources and Life Science Vienna).

FIGURE 24

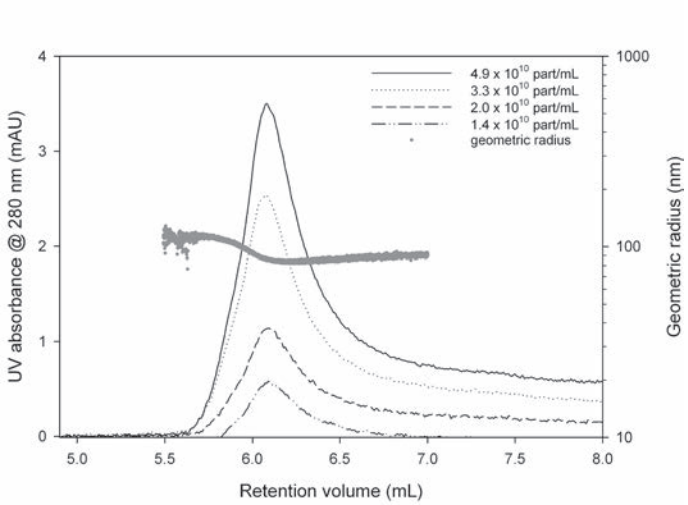
ANALYSIS OF POLYSACCHARIDES



Columns:	TSKgel G6000PW + G4000PW, two 7.5 mm ID × 60 cm L columns in series
Mobile phase:	H_2O with 0.2 mol/L NaCl
Flow rate:	0.9 mL/min
Temperature:	40 °C
Sample:	hyaluronic acid, polyethylene oxide

FIGURE 25

SEC PEAKS OF DIFFERENT HIV-1 gag VLP CONCENTRATIONS



Column:	TSKgel G5000PWXL with TSKgel PWXL guard column
Mobile phase:	25 mM Na-phosphate, 250 mM NaCl
Flow rate:	0.3 mL/min
Temp.:	25 °C
Detection:	UV @ 280 nm (A); MALS (B)
Injection vol.:	25 µL
Sample:	HIV-1 gag VLP (1.4×10^{10} - 6.5×10^{10} part/mL)

SEC/GFC
ABOUT TSKgel SuperMultiporePW

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.

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 50 for additional information). Each particle, by design, has an extended linear calibration curve, thereby greatly diminishing the inflection points on chromatograms. This allows better reproducibility when determining molecular mass and molecular mass distribution of polymers. Three semi-micro columns are available within the TSKgel SuperMultiporePW series (Figure 26).

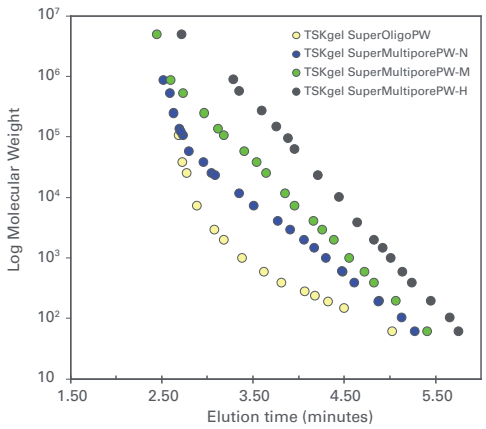
Multi-pore, semi-micro SEC columns provide high resolution and smooth peak shapes without shoulders of inflection points. This leads to better accuracy and reproducibility when analyzing water soluble polymers.

COMPARISON WITH CONVENTIONAL GPC COLUMNS

Figure 27 shows the analysis of Polyvinylpyrrolidone (PVP) K-30 on a series of conventional TSKgel G3000PW$_{XL}$ and G5000PW$_{XL}$ columns compared to the one obtained with a single SuperMultiporePW-M linear column (MW range 600,000 – 1,500,000). On the series of conventional columns the PVP K-30 peak shows an inflection point, which does not appear on SuperMultiporePW-M. Analysis is much faster and more sensitive when applying the multi-pore packing.

FIGURE 26

CALIBRATION CURVES OF POLYETHYLENE GLYCOL, OXIDE AND ETHYLENE GLYCOL

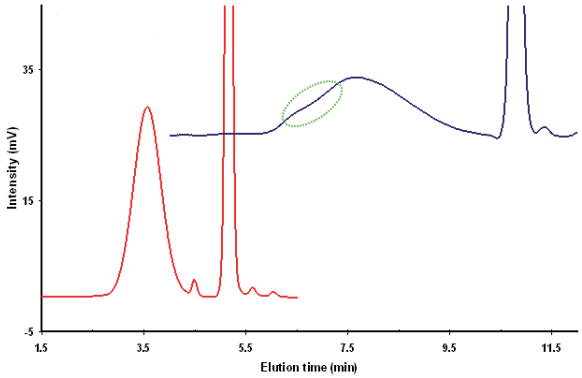


Columns:	TSKgel SuperOligoPW, SuperMultiporePW-N, SuperMultiporePW-M, SuperMultiporePW-H (each 6.0 mm ID x 15 cm L)
Mobile phase:	H_2O
Flow rate:	0.60 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	polyethylene oxides (PEO) standards, polyethylene glycols (PEG) standards, ethylene glycol (EG) standards

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 G3000PW$_{XL}$ and G5000PW$_{XL}$ columns in series. The analysis using the TSKgel SuperMultiporePW-M column was completed in half the time and with higher resolution than the analysis performed using conventional columns (Figure 28).

FIGURE 27

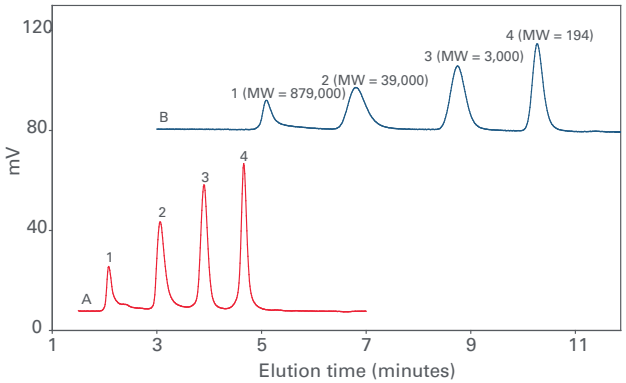
ANALYSIS OF POLYVENYLPYRROLIDONE



Columns:	TSKgel SuperMultiporePW-M, 6 mm ID x 15 cm L x 1 (red) TSKgel G3000PW$_{XL}$ & G5000PW$_{XL}$, each 7.8 mm ID x 30 cm L in line (blue)
Mobile phase:	0.1 mol/L $NaNO_3$
Flow rate:	0.6 mL/min
Detection:	RI
Sample:	Polyvinylpyrrolidone (K-30)

FIGURE 28

COMPARISON OF ANALYSIS OF A MIXTURE OF PEO AND PEG



Resolution	TSKgel PW$_{XL}$	TSKgel SuperMultiporePW-M
Peak 1/Peak 2	3.45	4.25
Peak 1/Peak 2	3.29	3.17
Peak 1/Peak 2	3.30	3.39

Column:	TSKgel SuperMultiporePW-M, 6.0 mm ID x 15 cm L TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$, 6.0 mm ID x 15 cm L
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Injection vol.:	A: 20 µL, B: 100 µL
Samples:	mixture of PEO and PEG

SEC/GFC
TSKgel PW FOR SPECIFIC APPLICATIONS

TSKgel SuperOligoPW AND G-Oligo-PW

The TSKgel SuperOligoPW semi-micro column featuring a small particle size has been designed for fast analysis of oligomers, particularly oligosaccharides, and low molecular weight aqueous polymers. It is a semi-micro column packed with monodisperse 3 µm polymethacrylate particles. The combination of the decreased particle size and small dimensions of the TSKgel SuperOligoPW column enables high-speed separation with high resolution. An added benefit of the semi-micro and small particle size is lower solvent consumption.

TSKgel G-Oligo-PW was designed for 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.

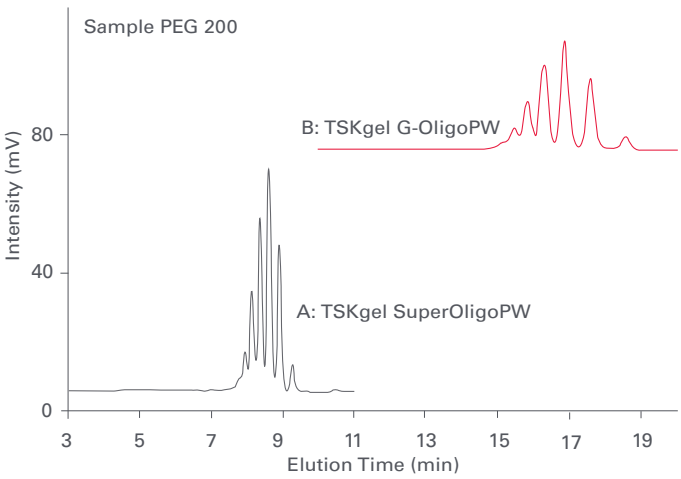
Since the packing material in the TSKgel SuperOligoPW columns is more hydrophilic compared with TSKgel G-Oligo-PW columns, an even wider range of water-soluble polymers can be analyzed without the need to add organic solvent to the eluent.

THE INFLUENCE OF PARTICLE SIZE

The influence of particle size on resolution and analysis time can be seen in Figure 29. It compares the separation of PEG 200 on two TSKgel G-Oligo-PW columns in series with 7 µm beads and two TSKgel SuperOligoPW semi-micro columns with a 3 µm material. At half of the analysis time an excellent resolution of the PEG 200 was obtained with the smaller particles in the TSKgel SuperOligoPW column.

FIGURE 29

ANALYSIS OF PEG 200. COMPARISON BETWEEN TSKgel SuperOligoPW AND TSKgel G-Oligo-PW



Column:	A. TSKgel SuperOligoPW, 6.0 mm ID x 15 cm L x 2
	B. TSKgel G-Oligo-PW, 7.8 mm ID x 30 cm L x 2
Mobile phase:	H_2O
Flow rate:	A: 0.6 mL/min, B: 1.0 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	A: 20 µL, B: 100 µL

SEC/GFC
TSKgel PW FOR SPECIFIC APPLICATIONS

TSKgel G-DNA-PW

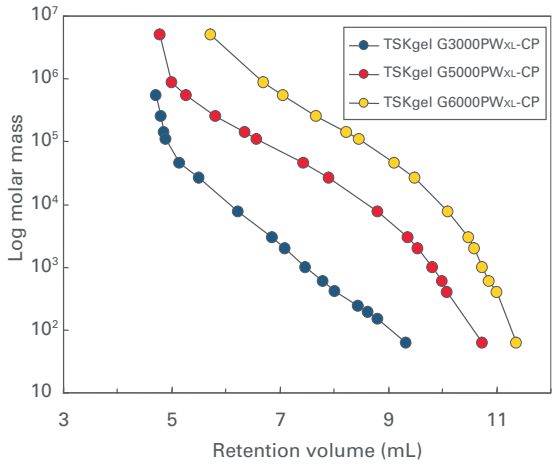
The TSKgel G-DNA-PW column is dedicated to the separation of large polynucleotides, such as DNA and RNA fragments of 500 to 5,000 base pairs. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs. This column is a smaller particle size version of the TSKgel G6000PWXL column. It has very large pores (> 100 nm) and a particle size of 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.

TSKgel PWXL-CP

TSKgel PWXL-CP columns are designed to facilitate the separation of cationic polymers by SEC at low salt conditions. Cationic surface modification of the PW packing 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 different ranges (G3000-, G5000- and G6000PWXL-CP). Figure 30 shows the calibration curves and Figure 31 shows the analysis of various cationic polymers on a series of TSKgel PWXL-CP columns.

FIGURE 30

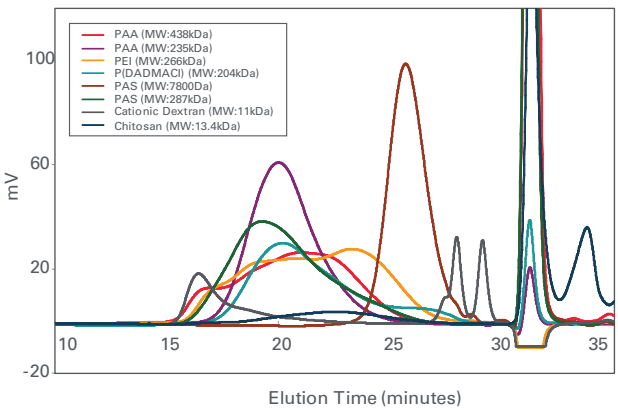
POLYETHYLENE GLYCOL AND OXIDE CALIBRATION CURVES FOR TSKgel PWXL-CP COLUMNS



Columns: TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm L
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm L
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID × 30 cm L
Mobile phase: H_2O with 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 31

ANALYSIS OF CATIONIC POLYMERS



Columns: TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID x 30 cm L
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID x 30 cm L
TSKgel G6000PWXL-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
Sample Load: 3 g/L, 100 µL

SEC/GFC
ORDERING INFORMATION TSKgel PW SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel PW Columns						
0005761	G2000PW	7.5	30	12	≥ 5,000	2.0
0005105	G2000PW	7.5	60	12	≥ 10,000	4.0
0008031	G-Oligo-PW	7.8	30	7	≥ 16,000	4.0
0022792	SuperOligoPW	6.0	15	3	>16,000	5.0
0008020	G2500PWXL	7.8	30	7	≥ 16,000	4.0
0008028	G2500PW	7.5	30	12	≥ 5,000	2.0
0008029	G2500PW	7.5	60	12	≥ 10,000	4.0
0008030	G2500PW	21.5	60	17	≥ 10,000	2.0
0008021	G3000PWXL	7.8	30	7	≥ 16,000	4.0
0021873	G3000PWXL-CP	7.8	30	7	≥ 16,000	5.5
0005762	G3000PW	7.5	30	12	≥ 5,000	2.0
0005106	G3000PW	7.5	60	12	≥ 10,000	4.0
0008022	G4000PWXL	7.8	30	10	≥ 10,000	2.0
0005763	G4000PW	7.5	30	17	≥ 3,000	1.0
0005107	G4000PW	7.5	60	17	≥ 6,000	2.0
0008023	G5000PWXL	7.8	30	10	≥ 10,000	2.0
0021874	G5000PWXL-CP	7.8	30	10	≥ 10,000	2.5
0005764	G5000PW	7.5	30	17	≥ 3,000	1.0
0005108	G5000PW	7.5	60	17	≥ 6,000	2.0
0008024	G6000PWXL	7.8	30	13	≥ 7,000	2.0
0021875	G6000PWXL-CP	7.8	30	13	≥ 7,000	2.0
0005765	G6000PW	7.5	30	17	≥ 3,000	1.0
0005109	G6000PW	7.5	60	17	≥ 6,000	2.0
0008032	G-DNA-PW	7.8	30	10	≥ 10,000	2.0
0008025	GMPWXL	7.8	30	13	≥ 7,000	2.0
0008026	GMPW	7.5	30	17	≥ 3,000	1.0
0008027	GMPW	7.5	60	17	≥ 6,000	2.0
0022789	SuperMultiporePW-N	6.0	15	4	>16,000	4.5
0022790	SuperMultiporePW-M	6.0	15	5	>12,000	2.7
0022791	SuperMultiporePW-H	6.0	15	8	>7,000	0.9
PEEK						
0020024	BioAssist G6PW	7.8	30	17	≥ 3,000	10

SEC/GFC
ORDERING INFORMATION TSKgel PW SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	
Guardcolumns					
0008033	PW$_{XL}$ Guardcolumn	6.0	4.0	12	For 7.8 mm ID PW$_{XL}$ & G-DNA-PW (TSKgel G3000PW packing)
0021876	PW$_{XL}$-CP Guardcolumn	6.0	4.0	13	For 7.8 mm ID PW$_{XL}$-CP columns
0006763	PW-L Guardcolumn	7.5	7.5	12	For 7.5 mm ID G2000PW (TSKgel G2000PW packing)
0008034	Oligo Guardcolumn	6.0	4.0	13	For 7.8 mm ID G-Oligo-PW columns
0022796	SuperOligoPW Guardcolumn	4.6	3.5	3	
0006762	PW-H Guardcolumn	7.5	7.5	12	For 7.5 mm ID G2500PW through GMPW columns
0022793	SuperMP (PW)-N Guardcolumn	4.6	3.5	4	
0022794	SuperMP (PW)-M Guardcolumn	4.6	3.5	5	
0022795	SuperMP (PW)-H Guardcolumn	4.6	3.5	8	
0006758	PW-H Guardcolumn	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

SEC
ABOUT TSKgel Alpha AND SuperAW

TSKgel Alpha and SuperAW column series offer a broad application range:

- The unique base matrix is compatible with many aqueous and organic solvents
- Six different pore sizes span a wide molecular weight separation range
- Small particle, semi-micro columns reduce analysis time and increase resolution

TSKgel Alpha AND SuperAW SERIES PROPERTIES

TSKgel Alpha and SuperAW columns were developed for the SEC analysis of polymers of intermediate polarity. As in the TSKgel PW and PWXL columns, the particles in these TSKgel columns have a hydroxylated methacrylate polymer backbone, but they differ in that they are cross-linked to a higher degree to minimize swelling in polar organic solvents (methanol, acetonitrile, DMSO, isopropanol, THF, and HFIP).

The TSKgel Alpha and SuperAW columns provide accurate molar mass determination and exhibit normal retention of polystyrene polymers in dimethyl formamide (DMF) solvent. Unlike TSKgel PW columns, which are stable to a 50% organic mixed with water at most, TSKgel Alpha and SuperAW columns are stable in a wide variety of organic solvents at concentrations up to 100%.

TSKgel Alpha and SuperAW columns are offered in five discrete exclusion ranges and as a mixed bed column. Product attributes of the TSKgel Alpha and SuperAW columns are shown in Table VI. These columns are for the analysis of polymers that are soluble in methanol, acetonitrile, DMSO, isopropanol, or THF and can also be used for water-soluble polymers.

TABLE VI

PROPERTIES AND SEPARATION RANGES FOR TSKgel PW-TYPE PACKINGS

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	5×10^3	1×10^4	1×10^4
Alpha-3000	7	9×10^4	1×10^5	6×10^4
Alpha-4000	10	4×10^5	1×10^6	3×10^6
Alpha-5000	10	1×10^6	7×10^6	N.D.
Alpha-6000	13	$> 1 \times 10^7$	$> 1 \times 10^7$	N.D.
Alpha-M	13	$> 1 \times 10^7$	$> 1 \times 10^7$	N.D.
SuperAW2500	4	5×10^3	8×10^3	1×10^4
SuperAW3000	4	9×10^4	8×10^4	1×10^5
SuperAW4000	6	1×10^6	6×10^5	6×10^5
SuperAW5000	7	1×10^{6}*	N.D.	N.D.
SuperAW6000	9	1×10^{7}*	N.D.	N.D.
SuperAWM-H	9	1×10^{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
TSKgel Alpha/SuperAW COLUMN SELECTION

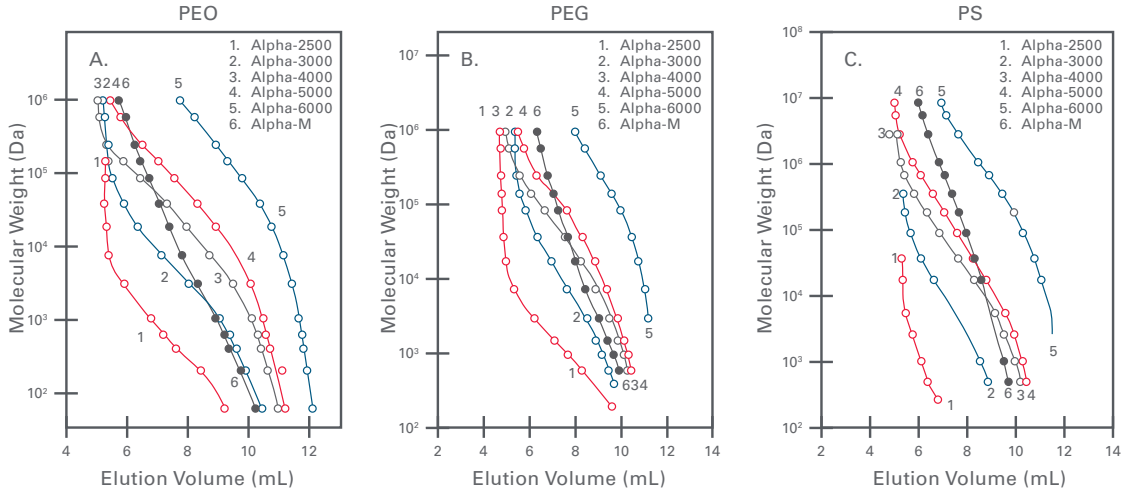
Use TSKgel Alpha columns when throughput is not critical, when sample mass is not limited, to collect fractions, and to obtain maximum number of plates (at the expense of analysis time). The main application area for TSKgel Alpha columns is the analysis of polymers that are soluble in polar organic solvents. Examples include cellulose derivatives, polyimide, and sodium dodecylsulfate (all in 10 mmol/L LiBr in DMF), cleansing gel in methanol, and degree of saponification of polyvinylalcohol in hexafluoroisopropanol (HFIP). The TSKgel Alpha Series consists of six columns. These columns span a wide molar mass separation range, from 100 to more than 1 × 10^6 Da, when using polyethylene oxide (PEO) as a molar mass standard. There is one mixed bed column within the TSKgel Alpha line, TSKgel Alpha-M, which has an extended linear calibration range and is suitable for samples with a broad molar mass distribution, as well as samples with unknown molar mass.

Use TSKgel SuperAW columns for high throughput applications, to reduce solvent consumption and to reduce solvent disposal cost. TSKgel SuperAW columns contains a similar chemistry as the TSKgel Alpha columns but offer the benefit of smaller particle sizes, smaller column dimensions, and equivalent resolution. Reductions in analysis time and mobile phase consumption make TSKgel SuperAW columns ideal for high throughput applications. The TSKgel SuperAW column line consists of five columns and a mixed bed column. These high efficiency columns are available in 6.0 mm ID × 15 cm dimensions.

Figures 32 and 33 show the calibration curves for the TSKgel Alpha and SuperAW 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 32

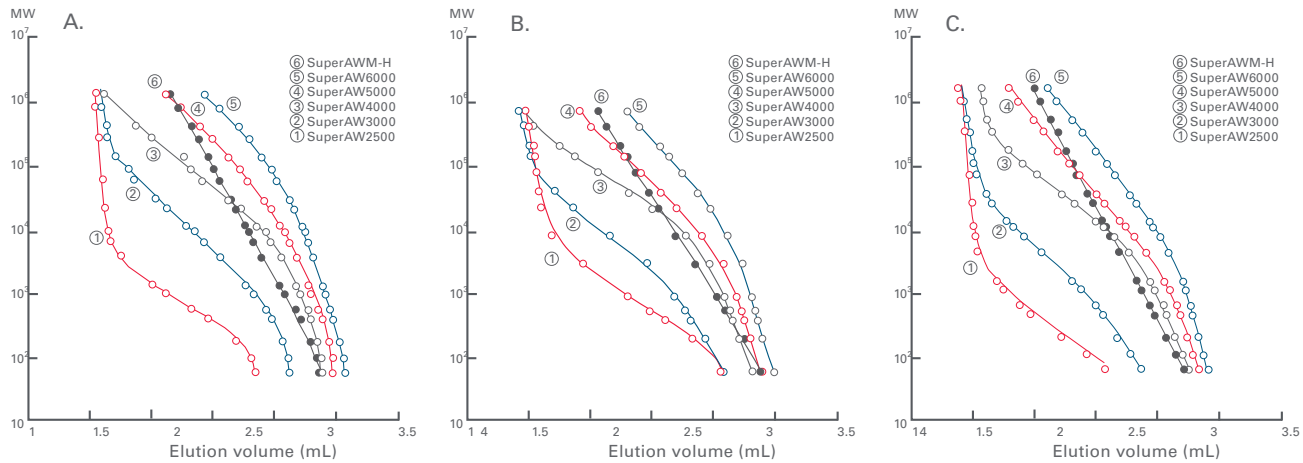
POLYETHYLENE OXIDE (PEO), POLYETHYLENE GLYCOL (PEG) AND POLYSTYRENE (PS) CALIBRATION CURVES FOR TSKgel Alpha COLUMNS



Column: TSKgel Alpha Series, 7.8 mm ID x 30 cm L; Mobile phase: 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

FIGURE 33

CALIBRATION CURVES FOR TSKgel SuperAW SERIES IN DIFFERENT SOLVENTS WITH DIFFERENT POLARITY



Column: TSKgel SuperAW Series (6.0 mm ID x 15 cm L); Mobile phase: 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

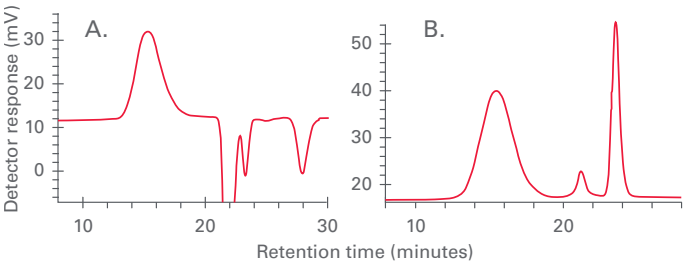
SEC
TSKgel Alpha AND Super AW APPLICATIONS

CELLULOSE DERIVATIVES

The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in Figure 34 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.

FIGURE 34

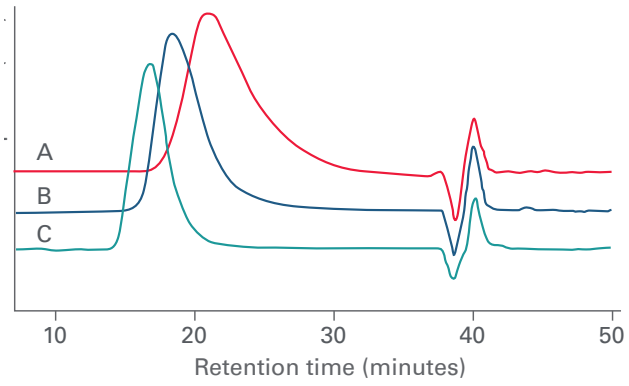
ANALYSIS OF CELLULOSE DERIVATIVES



Column:	TSKgel Alpha-M, 13 µm, 7.8 mm ID × 30 cm
Mobile phase:	A. DMF with 10 mmol/L LiBr
	B. MeOH with 10 mmol/L LiBr
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	50 µL
Samples:	A. ethyl cellulose, 0.1%
	B. ethyl hydroxyethyl cellulose, 0.1%

FIGURE 35

ANALYSIS OF POLYVINYLALCOHOL WITH DIFFERENT DEGREES OF SAPONIFICATION



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

POLYVINYLALCOHOL CHARACTERIZATION

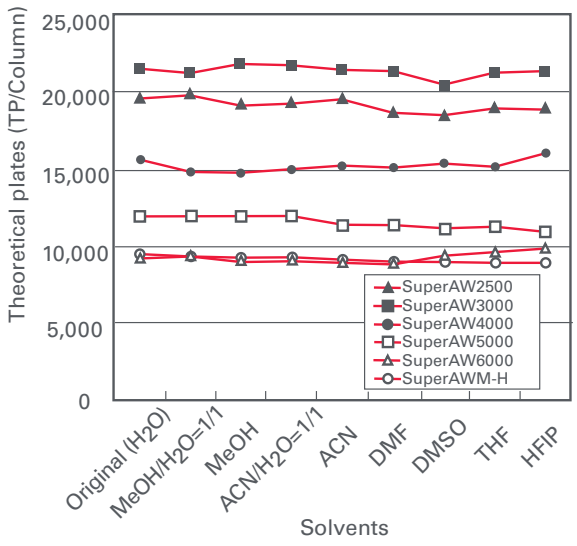
The separation of polyvinylalcohol with different degrees of saponification is shown in Figure 35. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol (HFIP) mobile phase.

SOLVENT COMPATIBILITY

As shown in Figure 36, efficiency of all TSKgel SuperAW columns is maintained when changing solvents from water via acetonitrile, DMF, DMSO, THF to HFIP.

FIGURE 36

COLUMN EFFICIENCY OF TSKgel SuperAW COLUMNS



Column:	TSKgel SuperAW columns, 6.0 mm ID × 15 cm L
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	5 µL (2.5 g/L)
Sample:	ethylene glycol

SEC
ORDERING INFORMATION TSKgel Alpha/Super AW

ORDERING INFORMATION

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

Shipping solvent in Alpha and SuperAW columns is water.

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

ORGANIC SEC
GEL PERMEATION CHROMATOGRAPHY

Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC) that separates molecules according to their hydrodynamic volume which is related to their molecular weight. The separation is based strictly on the size of the sample in solution, and there should be no interaction with the stationary phase of the GPC column.

Elution order in GPC is that of an "inverse-sieving" technique, large molecules access a smaller pore volume than smaller molecules resulting in larger molecules eluting from the GPC column prior to the smaller molecules.

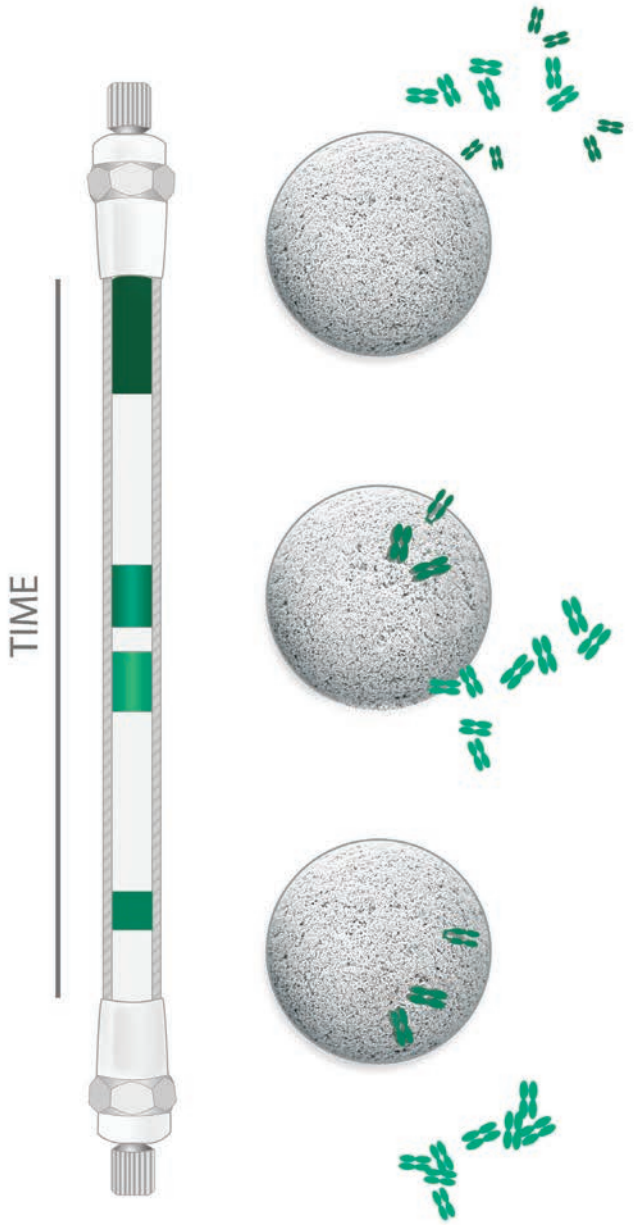
GPC can determine several important parameters. These include number average molecular weight (Mn), weight average molecular weight (Mw), Z weight average molecular weight (Mz), and the most fundamental characteristic of a polymer, its molecular weight distribution. These parameters are important, since they affect many of the characteristic physical properties of a polymer. Differences in these parameters can cause significant differences in the end-use properties of a polymer.

GPC/SEC is usually carried out at room temperature, but some polymers such as high molecular weight polyolefins need high temperature for effective dissolution. Hence, GPC analysis of these polymers needs to be performed at higher temperature.

GPC plays an important role in the characterization of polar organic-soluble and organic-soluble polymers in consumer, chemical, and petrochemical industries.

FIGURE 37

GEL PERMEATION CHROMATOGRAPHY ILLUSTRATION



SEC/GPC - INSTRUMENTS

The experience gained from more than 40 years of Gel Permeation Chromatography (GPC) instrumentation development is clearly visible in the All-In-One System architecture of the EcoSEC GPC System. This design concept is the foundation on which the benefits of the system rest: low dead volume for improved resolution and molar mass distribution accuracy, temperature controlled pumps for excellent flow rate precision regardless of changes in laboratory temperature, and dual flow RI (refractive index) detection for unmatched baseline stability.

Time and solvent can be saved using the EcoSEC GPC System with optional semi-micro columns due to the system's low dead volume. The dead volume of the EcoSEC GPC System (< 20 µL) is less than half the dead volume of conventional GPC systems.

Application area:
Organic-soluble polymers
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

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.

ALL-IN-ONE-SYSTEM

Superior performance

- Unmatched baseline stability due to unique dual flow RI detector design
- High degree of precision in retention time and molar mass determination due to advanced temperature controlled pumps
- Exceptional reproducibility day to day, system to system, and site to site

Increased throughput

- Stable RI baseline with low baseline drift in THF obtained within 90 minutes of start up
- Unattended operation with built-in autosampler

Unparalleled versatility

- Column switching valve reduces time between column changes and rapidly establishes a stable baseline (within 15 minutes)
- Easy to use, intuitive software specific to GPC analysis
- Optional UV detector for measurement of UV-absorbing polymers
- Compatible with external viscometry and multi-angle light scattering detectors

Optional semi-micro columns

- 50% reduction in run times and solvent cost savings of 85% due to low dead volume design
- TSKgel SuperMultiporeHZ columns are packed with particles synthesized with a range of pore sizes, resulting in no inflection points in the calibration curve. The lack of inflection points allows better accuracy and reproducibility when determining the molar mass distribution of polymers.

SEC/GPC
ABOUT TSKgel H SERIES

TSKgel GPC column highlights

- Porous, highly crosslinked polystyrene divinylbenzene (PS-DVB) particles
- Expanded molecular weight ranges
- Semi-micro column dimensions for reduced solvent consumption and fast analysis
- Proprietary multi-pore technology for extended linear range
- High temperature GPC columns for use up to 220 °C

TSKgel H SERIES PROPERTIES

TSKgel H series columns are recommended for the analysis of organic-soluble polymers and are packed with spherical particles composed of polystyrene crosslinked with divinylbenzene (PS-DVB). This series includes TSKgel HXL, HHR, SuperH, SuperHZ, and SuperMultiporeHZ columns. Each line of columns within this series differs in degree of inertness and operating temperature range.

The Super prefix designates short (15 cm) columns packed with particles as small as 3 µm. The smaller particle allows for equivalent resolution to conventional TSKgel HXL columns, with 50% reduction in analysis time due to the shorter column length. The TSKgel Super series columns are an excellent choice for high throughput polymer analysis.

A comparison of TSKgel H series columns is detailed in Table VI. Best results are obtained when selecting a column with the sample's molar mass in the linear portion of the calibration curve.

The cross-linking of the polystyrene particles in TSKgel H series columns ensures minimal shrinking and swelling of the column bed when the organic solvent is changed according to the solvent recommendations outlined in Table VII.

Suggested flow rates for TSKgel SuperH and HHR columns are outlined in Table VIII. Table IX lists the recommended solvents by application for TSKgel H series columns.

TABLE VI

PROPERTIES OF TSKgel GPC COLUMNS

TSKgel series	SuperMultiporeHZ	SuperHZ	SuperH	HXL	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	High throughput polymer analysis with expanded solvent compatibility range	Conventional polymer analysis with ultra-low polymer adsorption, limited solvent compatibility range	Conventional polymer analysis with expanded solvent compatibility
Particle size	3 µm, 4 µm, and 6 µm, depending on pore size	3 µm, 5 µm, and 10 µm, depending on pore size	3 µm and 5 µm, depending on pore size	5 µm, 6 µm, 9 µm, and 13 µm, depending on pore size	5 µm, 13 µm, 20 µm, and 30 µm
Particle matrix	Polystyrene divinylbenzene (PS-DVB)				
Number of solvent substitutions	None	One time only	Several[1]	One time only	Several[1]

[1] *After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.*

SEC/GPC
TSKgel H SERIES MOBILE PHASE SELECTION

TABLE VII

SOLVENT COMPATIBILITY FOR TSKgel H SERIES COLUMNS

TSKgel series	Shipping solvent*	Can be replaced with:
SuperHZ and H_{XL}[1]	Tetrahydrofuran[3,4]	benzene, chloroform, toluene, xylene, dichloromethane, dichloroethane
	Acetone**	carbon tetrachloride[5], o-dichlorobenzene, dimethylformamide, dodecane, dimethyl sulfoxide, dioxane, ethylacetate, FC-113, hexane, pyridine, hexafluoroisopropanol/chloroform, methyl ethyl ketone, quinoline, cyclohexane
	Chloroform**	m-cresol in chloroform, up to 10% hexafluoroisopropanol/chloroform
	Dimethylformamide	dimethyl sulfoxide, dioxane, tetrahydrofuran, toluene
SuperH and H_{HR}[2]	Tetrahydrofuran[3]	acetone, ethanol, quinoline, benzene, o-dichlorobenzene, ethyl acetate, dodecane, FC-113, carbon tetrachloride[5], dichloromethane, dichloroethane, trichloroethane, n-hexane, cyclohexane, xylene, tetrahydrofuran, chloroform, 1,4-dioxane, hexafluoroisopropanol, toluene, 1-chloronaphthalene, N,N-dimethylacetoacetamide, methyl ethyl ketone, trichlorobenzene, m-cresol, dimethylformamide, methylpyrrolidone, o-chlorophenol/chloroform, dimethyl sulfoxide, pyridine
SuperMultiporeHZ	Tetrahydrofuran[3]	Cannot be replaced. TSKgel SuperMultiporeHZ columns can be used only in tetrahydrofuran.

[1] In case of TSKgel SuperHZ and H_{XL}, keep flow rate as mentioned below during solvent change. Solvent can be changed one way/one time only. TSKgel H_{XL}: below <0.5 mL/min; TSKgel SuperHZ (4.6 mm ID): below <0.15 mL/min; TSKgel SuperHZ (6.0 mm ID): below <0.3 mL/min

[2] In case of TSKgel SuperH and H_{HR}, see Table 22 below for appropriate flow rates for solvent exchange. After switching to a very polar solvent, switching to a nonpolar solvent is not recommended.

[3] All TSKgel H_{XL}, H_{HR}, SuperHZ, SuperH, SuperMultipore, and GMH analytical columns are shipped containing tetrahydrofuran (THF), except the TSKgel high temperature columns, which contain o-dichlorobenzene (ODCB).

[4] THF in TSKgel G1000H_{XL} columns cannot be replaced with dichloromethane or dichloroethane.

[5] Prolonged exposure to carbon tetrachloride can corrode the stainless steel parts of a column and an HPLC system.

** 100% methanol cannot be used with TSKgel H series columns; use this solvent with TSKgel SW or Alpha columns.*

*** TSKgel H series columns may be specially ordered with this shipping solvent.*

TABLE VIII

RECOMMENDED FLOW RATES (mL/min) FOR TSKgel SuperH AND H_{HR} COLUMNS

Solvent	TSKgel SuperH 6.0 mm ID × 15 cm L	TSKgel H_{HR} 7.8 mm ID × 30 cm L
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 IX

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

SEC/GPC MULTIPORE TECHNOLOGY

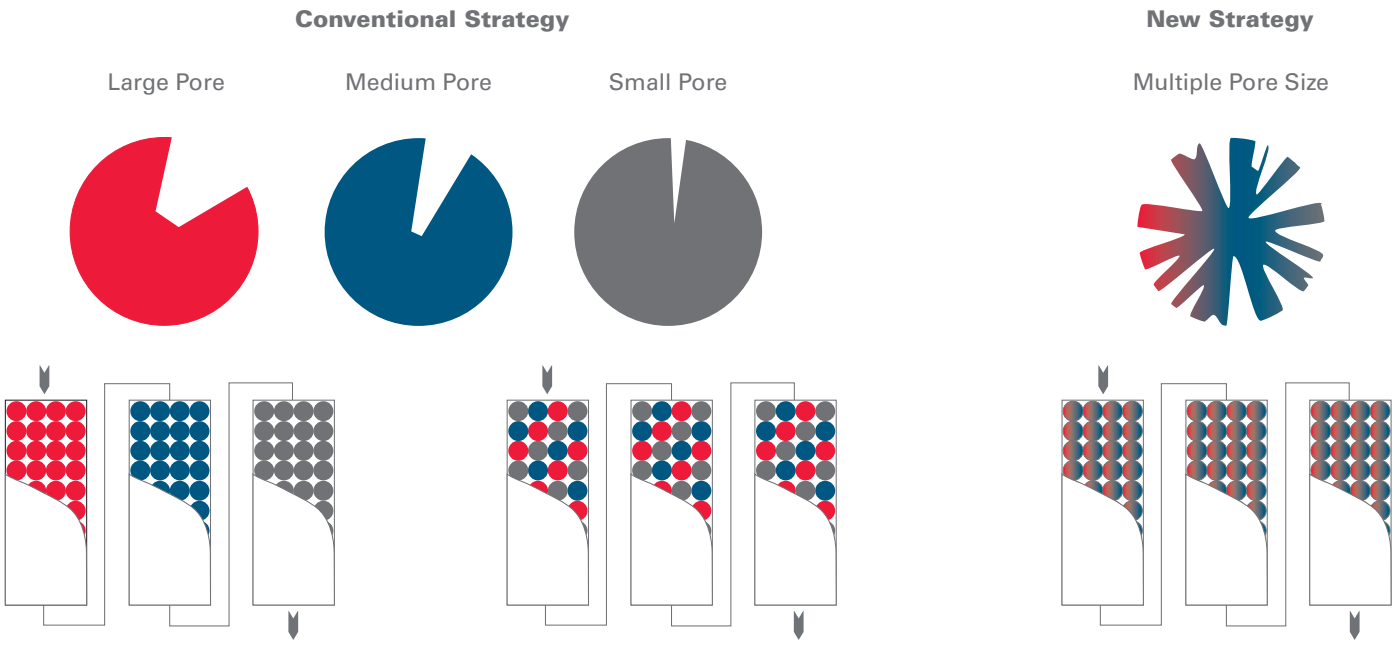
The innovative Multipore approach, pioneered by Tosoh, is a synthetic chemistry answer to the question of how to obtain a column with an extended linear calibration curve, while mixed bed columns represent a mechanical way of obtaining a linear calibration curve. In general, Multipore columns have a smoother, more linear, calibration curve.

Prior to the introduction of TSKgel Multipore and SuperMultipore 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 of different pore size resins at an optimized mix ratio. However, problems can occur with both of these methods e.g. distortion of the chromatogram.

As is shown in Figure 38, a novel approach to solve this problem was developed by Tosoh scientists and is incorporated in TSKgel MultiporeHXL and SuperMultiporeHZ Series columns. These columns are packed with particles of uniform size synthesized with a broad distribution of pore sizes. This creates a linear calibration curve within each particle. Columns with an extended linear calibration curve can 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.

FIGURE 38

STRATEGIES FOR WIDE RANGE SEPARATION USING SEC



SEC/GPC
MULTIPORE TECHNOLOGY

The pore size distributions of the TSKgel MultiporeHXL-M column and a mixed-bed column are shown in Figure 39. 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.

The small ID (4.6 mm) and length (15 cm) of the Super-MultiporeHZ columns reduces solvent consumption and results in quick run times, and offers high throughput capabilities. Figure 40 demonstrates that inflection points are no longer observed with semi-micro columns packed from particles prepared by Multipore technology.

FIGURE 39

PORE SIZE DISTRIBUTION OF TSKgel MultiporeHXL-M COLUMN AND A MIXED-BED COLUMN

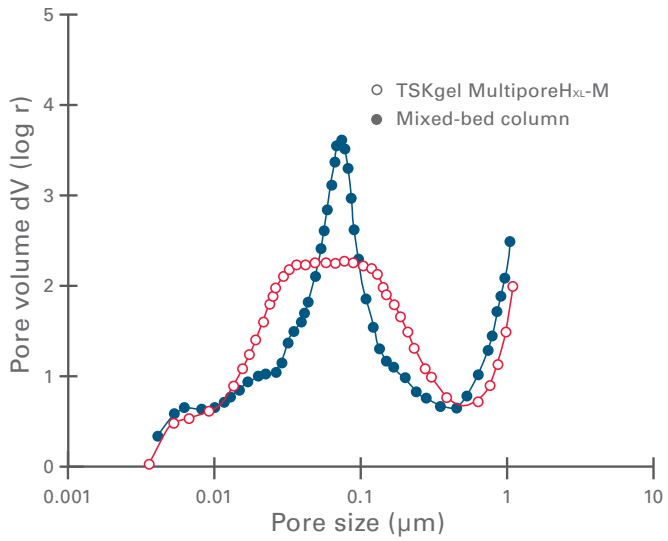
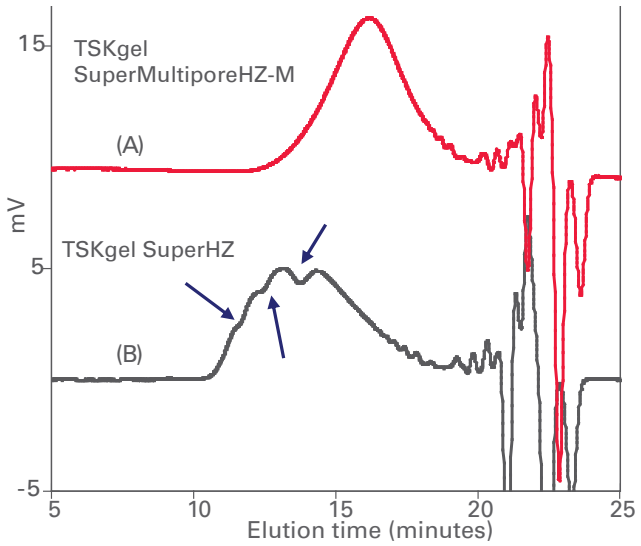


FIGURE 40

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

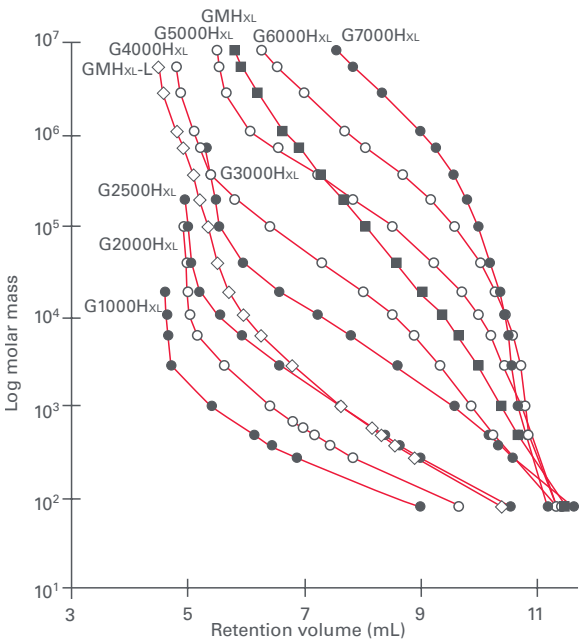
SEC/GPC
ABOUT TSKgel HXL

TSKgel HXL columns are conventional GPC columns of 7.8 mm ID × 30 cm containing particles composed of PS-DVB. The TSKgel HXL column lines consists of eight columns with different pore sizes (TSKgel G1000HXL through TSKgel G7000HXL) and three columns with an extended linear range of the calibration curve (TSKgel GMHXL, TSKgel GMHXL-L and TSKgel MultiporeHXL-M). The TSKgel GMHXL and TSKgel GMHXL-L linear columns are mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The remaining column, TSKgel MultiporeHXL-M, is a multi-pore column, in which each particle contains a range of pore sizes that provide a linear calibration curve.

TSKgel HXL columns are for the use of conventional polymer analysis and show ultra-low polymer absorption, i.e., the columns show true size exclusion behavior for most polymers. TSKgel HXL columns are shipped in THF, with the exception of the TSKgel GMHXL HT column, which is shipped in o-dichlorobenzene. These columns can be exchanged for a limited number of organic solvents. See Table IX for a listing of these solvents. Figures 41-42 show the calibration curves for the TSKgel HXL columns.

FIGURE 41

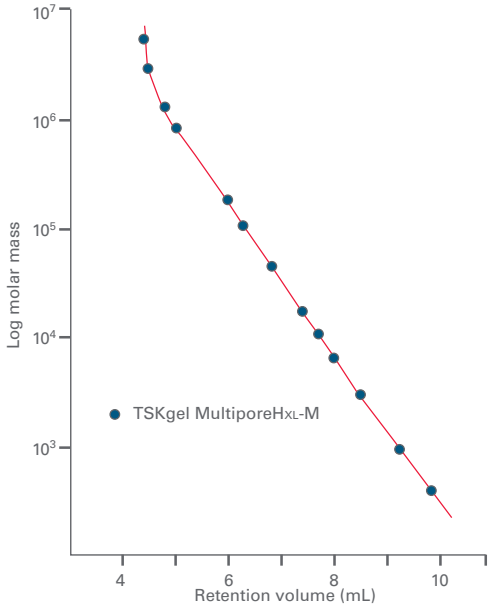
CALIBRATION CURVES OF TSKgel HXL COLUMNS



Column: TSKgel HXL columns, 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 standards

FIGURE 42

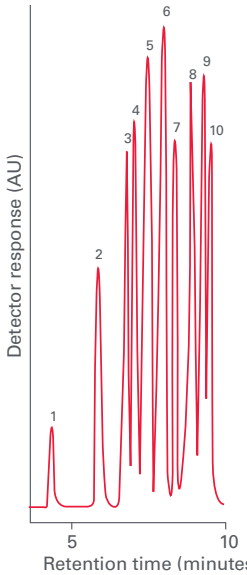
CALIBRATION CURVE OF TSKgel MultiporeHXL-M COLUMN



Columns: TSKgel MultiporeHXL-M, 5 µm, 7.8 mm ID × 30 cm L
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40 °C
Sample: polystyrene standards

FIGURE 43

HIGH RESOLUTION OF PHTHALATE ESTERS



Column: TSKgel G1000HXL, 5 µm, 7.8 mm ID × 30 cm L
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Samples: 1. polystyrene (1.0 × 10⁴ Da) 2. dioctylphthalate (391 Da)
3. dibutylphthalate (278 Da) 4. diprophylphthalate (250 Da)
5. diethylphthalate (222 Da) 6. dimethylphthalate (194 Da)
7. n-propylbenzene (120 Da) 8. ethylbenzene (116 Da)
9. toluene (92 Da) 10. benzene (78 Da)

SEC/GPC
TSKgel HXL APPLICATIONS

PHTHALATE ESTERS

Figure 43 demonstrates the high efficiency separation on a TSKgel G1000HXL column for low molar mass phthalate esters. Separation was close to baseline even though the molar masses of the esters differed by less than 50 Da.

FATTY ACIDS

In Figure 44, two TSKgel G2000HXL columns in series separate a mixture of fatty acids ranging from C4 to C30.

Epoxy Resin

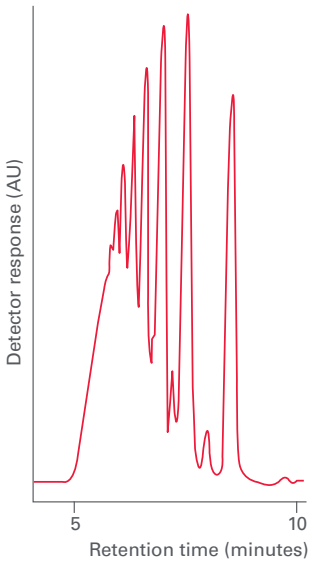
The analysis of a commercial epoxy resin, Epikote 1001, using a TSKgel G2500HXL column is shown in Figure 45.

ACRYLIC POLYMER

Figure 46 shows the separation of an acrylic polymer on the TSKgel MultiporeHXL-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 MultiporeHXL-M column.

FIGURE 45

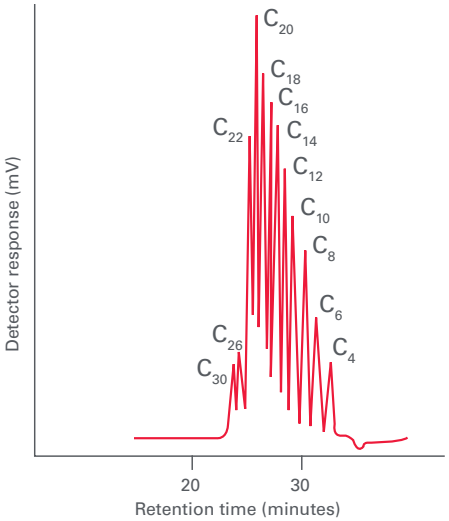
SEPARATION OF EPOXY RESIN



Column:	TSKgel G2500HXL, 5 µm, 7.8 mm ID × 30 cm L
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Sample:	Epikote 1001 epoxy resin

FIGURE 44

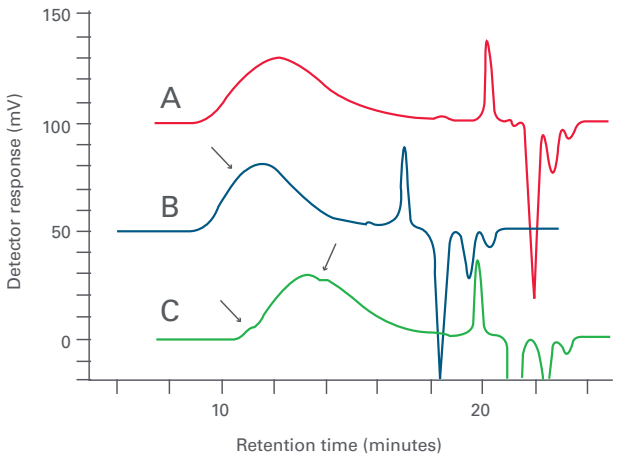
SEPARATION OF FATTY ACIDS



Column:	TSKgel G2000HXL, 5 µm, 7.8 mm ID × 30 cm L × 3
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	RI
Sample:	fatty acids

FIGURE 46

SEPARATION OF ACRYLIC RESIN



Columns:	A. TSKgel MultiporeHXL-M, 5 µm, 7.8 mm ID × 30 cm L × 2 in series B. Competitor P, 7.5 mm ID × 30 cm L × 2 in series, mixed bed type C. Competitor S, 8.0 mm ID × 30 cm L × 2 in series, mixed bed type
Mobile phase:	THF
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	RI
Sample:	acrylic polymer (0.1%, 50 µL)

SEC/GPC
ABOUT TSKgel HHR

TSKgel HHR columns are conventional GPC columns with dimensions of 7.8 mm ID × 30 cm containing particles composed of PS-DVB. The TSKgel HHR column line consists of eight columns with different pore sizes, TSKgel G1000HHR through TSKgel G7000HHR, and ten columns with an extended linear range of the calibration curve. Several columns of the TSKgel HHR Series (indicated by 'HT' or 'HT2' in the name) are suited for high temperature GPC analysis.

The mixed bed linear columns contain particles with different pore sizes that are blended to provide an extended linear calibration curve. They feature increasing linear calibration ranges, from TSKgel GMHHR-L, GMHHR-N, GMHHR-M, to GMHHR-H. All of the TSKgel high temperature mixed bed columns are shipped in ODCB (o-dichlorobenzene).

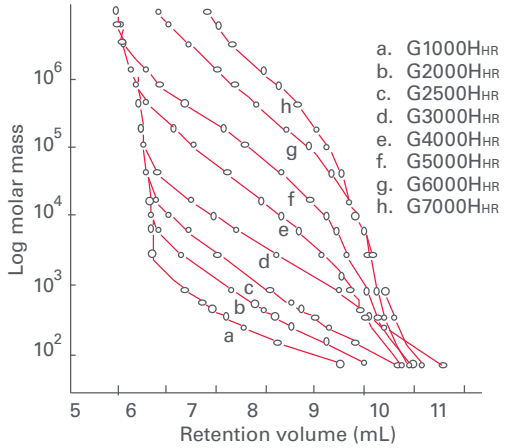
The TSKgel HHR HT2 mixed bed columns are available for ultra-high temperature analysis. Packed with PS-DVB beads, the maximum operating temperature of these columns is 220 °C.

The issue of shearing that occurs with the analysis of ultra-high molar mass polymers is overcome by the TSKgel GMHHR-M (S), TSKgel GMHHR-H (S), GMHHR-H (S) HT and GMHHR-H (S) HT2 columns. The (S) is a reference to this shearing effect.

TSKgel HHR columns have a broad solvent range and are shipped in THF, except for the high temperature mixed bed columns, which are shipped in ODCB. THF can be exchanged for a wide variety of organic solvents. See the table on page 49 for a listing of these solvents. Figures 47-49 show the calibration curves for the TSKgel HHR columns.

FIGURE 47

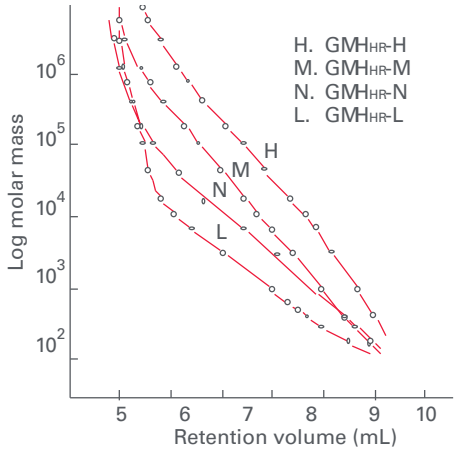
CALIBRATION CURVES OF TSKgel HHR COLUMNS



Column:	TSKgel HHR columns, 7.8 mm ID × 30 cm L
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	polystyrene standards

FIGURE 48

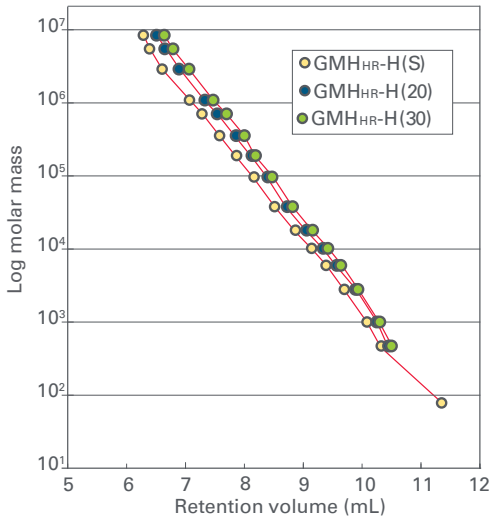
CALIBRATION CURVES OF TSKgel HHR MIXED BED COLUMNS



Columns:	TSKgel HHR columns, 7.8 mm ID × 30 cm L
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	polystyrene standards

FIGURE 49

CALIBRATION CURVES OF LINEAR TSKgel HHR COLUMNS



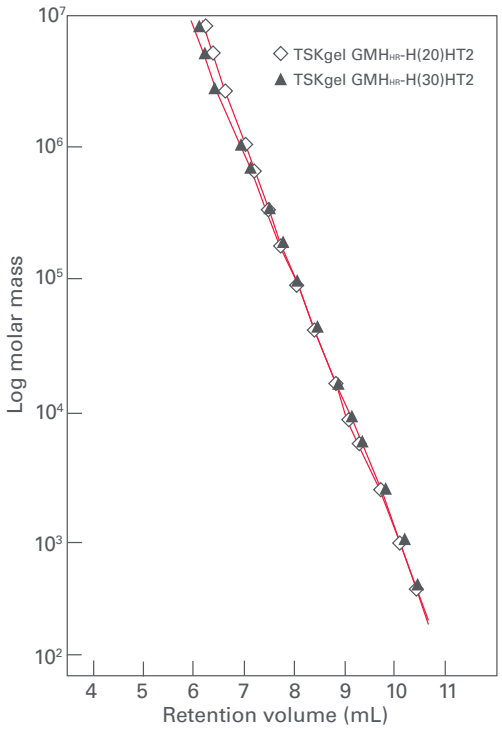
Columns:	TSKgel GMHHR-H (S), 13 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (20), 20 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (30), 30 µm, 7.8 mm ID × 30 cm L
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

SEC/GPC
ABOUT TSKgel HHR

Figures 50-51 show the calibration curves for the TSKgel HHR high temperature columns.

FIGURE 50

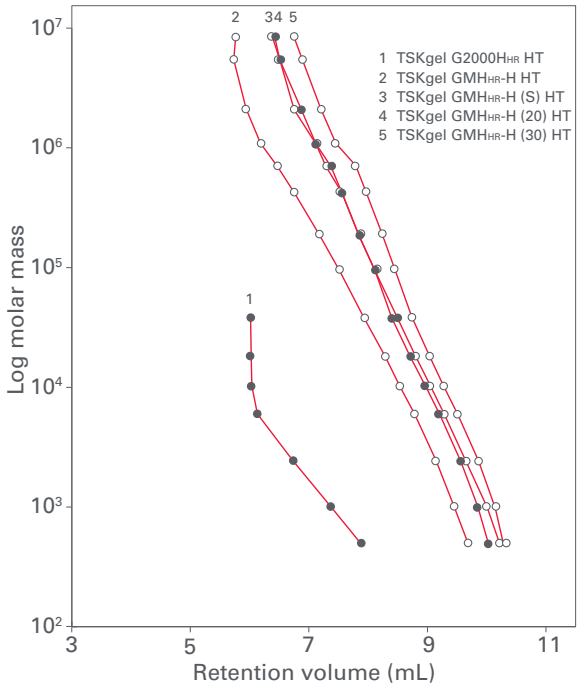
CALIBRATION CURVES OF TSKgel GMHHR-H HT2 COLUMNS



Columns:	TSKgel GMHHR-H (20) HT2, 20 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (30) HT2, 30 µm, 7.8 mm ID × 30 cm L
Mobile phase:	ODCB with 0.05% BHT
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	135 °C
Sample:	polystyrene standards

FIGURE 51

CALIBRATION CURVES OF TSKgel HT COLUMNS



Columns:	TSKgel G2000HHR (20) HT, 20 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H HT, 5 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (S) HT, 13 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (20) HT, 20 µm, 7.8 mm ID × 30 cm L
	TSKgel GMHHR-H (30) HT, 30 µm, 7.8 mm ID × 30 cm L
Mobile phase:	ODCB with 0.05% BHT
Flow rate:	1.0 mL/min
Detection:	RI (EcoSEC High Temperature GPC System)
Temperature:	135 °C
Injection vol.:	300 µL
Sample:	polystyrene

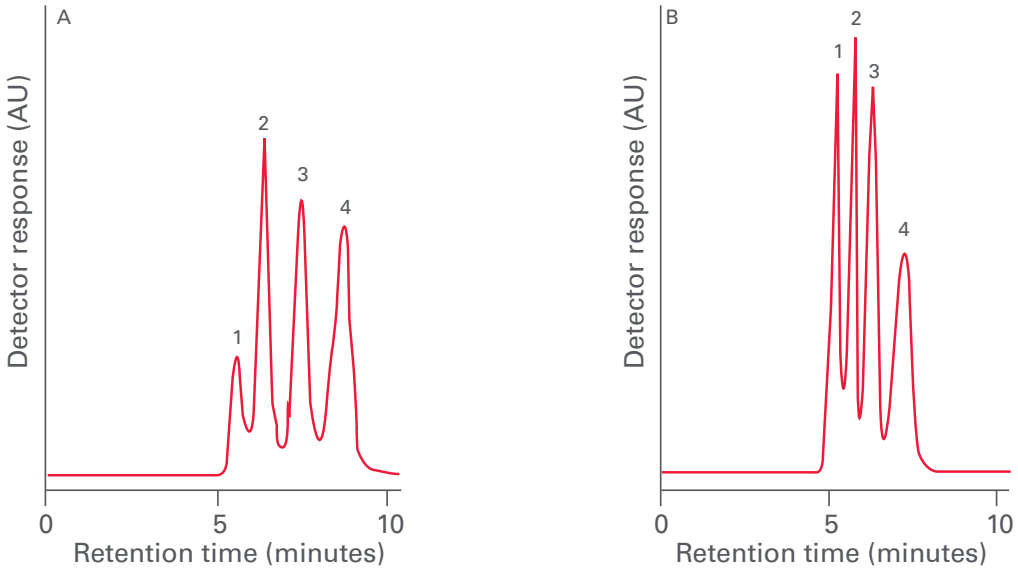
SEC/GPC
TSKgel HHR APPLICATIONS

POLYMETHYL METHACRYLATE

The effect of different pore size distributions in the mixed beds of TSKgel GMHHR-H and TSKgel GMHHR-M is illustrated in Figure 52. The TSKgel GMHHR-M produces sharper polymethyl methacrylate peaks in the 8.0×10^5 to 1.0×10^4 Da range.

FIGURE 52

COMPARISON OF STANDARD POLYMETHYL METHACRYLATE MIXTURE



Columns:	A. TSKgel GMHHR-H, 5 µm, 7.8 mm ID × 30 cm L
	B. TSKgel GMHHR-M, 5 µm, 7.8 mm ID × 30 cm L
Mobile phase:	5 mmol/L sodium trifluoroacetate in HFIP
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40 °C
Sample:	standard polymethylmethacrylate
	1. 8.2 × 105 Da, 2. 6.7 × 104 Da, 3. 1.02 × 104 Da, 4. 1,950 Da

SEC/GPC
HIGH TEMPERATURE GPC APPLICATIONS

HIGH TEMPERATURE GPC UP TO 220 °C

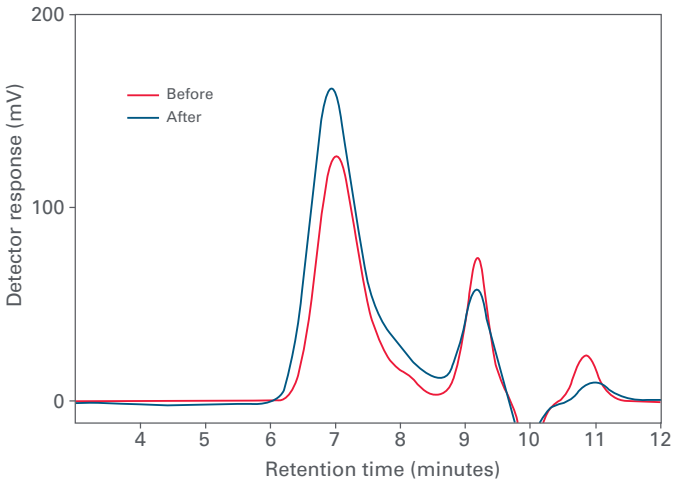
Column durability in high temperature GPC polymer analysis is essential as these columns are continuously exposed to harsh organic solvents, extremely elevated temperatures and temperature cycling as GPC systems are turned on and off. The durability of a high temperature GPC column directly influences the quality, applicability and selectivity, or resolution, of the GPC column, thus the accuracy of the molar mass averages obtained.

As a high temperature GPC column begins to fail or lose resolution due to the extreme experimental conditions required for high temperature GPC polymer analysis, the number- and z-average molar mass values obtained become inflated and the GPC elution profile begins to shift due to a decrease in multiple factors that affect the ability of the columns to separate species varying in hydrodynamic volume.

A durability and stability study of a TSKgel GMH$_{HR}$-H (S) HT high temperature GPC column was performed and the results were compared to another commercially available column for polymer analysis at 220 °C. The deterioration of the commercially available high temperature GPC column is observed in the GPC elution profiles, Figure 53, as the resolution between the sample and solvent peaks decreases after the column is exposed to temperature cycling. The GPC elution profiles obtained for the TSKgel GMH$_{HR}$-H (S) HT column before and after temperature cycling remain superimposable, Figure 54.

FIGURE 53

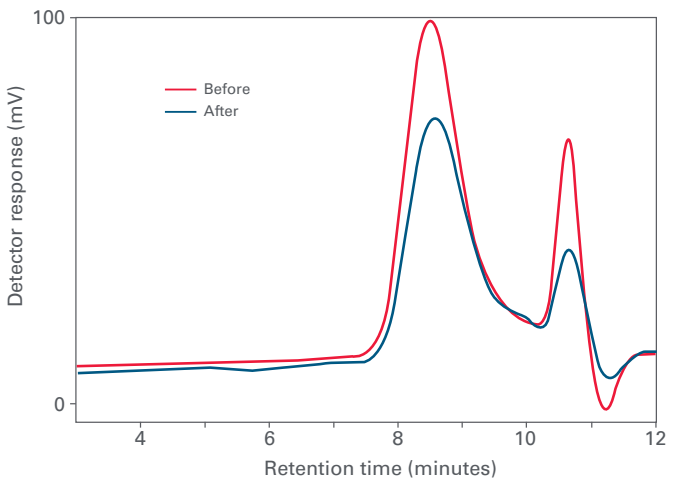
GPC ELUTION PROFILE FOR A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A COMMERCIALLY AVAILABLE HIGH TEMPERATURE GPC COLUMN



Column:	Commercially available high temperature GPC column, 13 µm, 7.8 mm ID × 30 cm L
Mobile phase:	1-chloronaphthalene
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	220 °C
Injection vol.:	200 µL
Sample:	synthetic polymer

FIGURE 54

GPC ELUTION PROFILE FOR A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A TSKgel GMH$_{HR}$-H (S) HT COLUMN



Column:	TSKgel GMH$_{HR}$-H (S) HT, 13 µm, 7.8 mm ID × 30 cm L
Mobile phase:	1-chloronaphthalene
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	220 °C
Injection vol.:	200 µL
Sample:	synthetic polymer

SEC/GPC
ABOUT TSKgel SuperH

TSKgel SuperH columns are conventional GPC columns with dimensions of 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB. The TSKgel SuperH column line consists of eight columns with different pore sizes, TSKgel SuperH1000 through TSKgel SuperH7000, and four columns with an extended linear range of the calibration curve.

TSKgel SuperH columns are high efficiency/high throughput versions of the conventional TSKgel HHR columns. Both column types are based on the same bead chemistry.

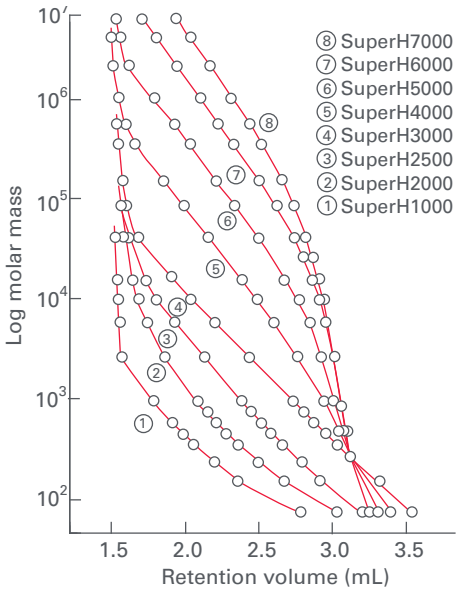
The TSKgel SuperH product line contains four mixed bed linear columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHM-L, SuperHM-M, SuperHM-N, to SuperHM-H.

The volume of a 6 mm ID × 15 cm TSKgel SuperH column is 3.4 times smaller than that of a conventional 7.8 mm ID × 30 cm column. As a result, peak volumes will be proportionally smaller on TSKgel SuperH columns compared to a corresponding TSKgel HHR column. Thus, your GPC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant. EcoSEC GPC systems are already optimized for these solvent saving applications.

The maximum operating temperature for TSKgel SuperH columns is 140 °C. All TSKgel SuperH columns are shipped in THF, which can be exchanged for a wide variety of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. Figures 55 and 56 show the calibration curves for the TSKgel SuperH columns.

FIGURE 55

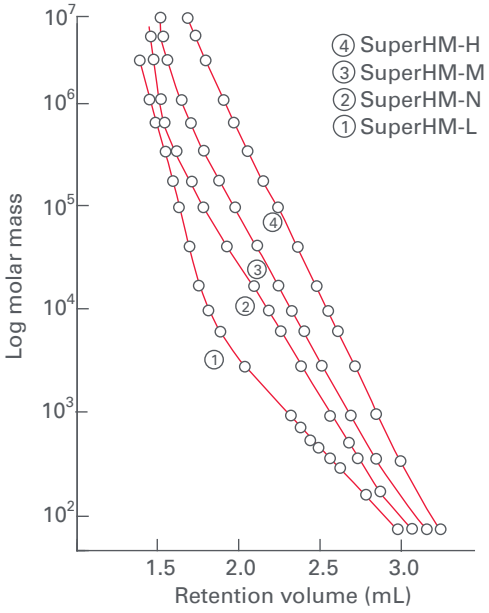
CALIBRATION CURVES FOR TSKgel SuperH COLUMNS



Column:	TSKgel SuperH columns, 6.0 mm ID × 15 cm L
Mobile phase:	THF
Flow rate:	0.6 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

FIGURE 56

CALIBRATION CURVES FOR TSKgel SuperH MIXED BED COLUMNS



Column:	TSKgel SuperH columns, 6.0 mm ID × 15 cm L
Mobile phase:	THF
Flow rate:	0.6 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

SEC/GPC
TSKgel SuperH APPLICATIONS

POLYSTYRENE MIXTURES

Figure 57 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperH2500 column with various organic solvents (THF, $CHCl_3$, DMF, and CCl_4) and Figure 58 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperHM-H column with various organic solvents.

Due to the interaction between the packing material and standard polystyrene when using DMF as the mobile phase, the elution volume of standard polystyrenes is greater than it is with "good" solvents such as THF and $CHCl_3$. This effect is particularly noticeable with TSKgel SuperH2500, a column for the analysis of low molar mass samples. Under these circumstances, polyethylene oxide (PEO) is recommended as the standard sample, as this reacts very little with the packing material.

COLUMN TEMPERATURE

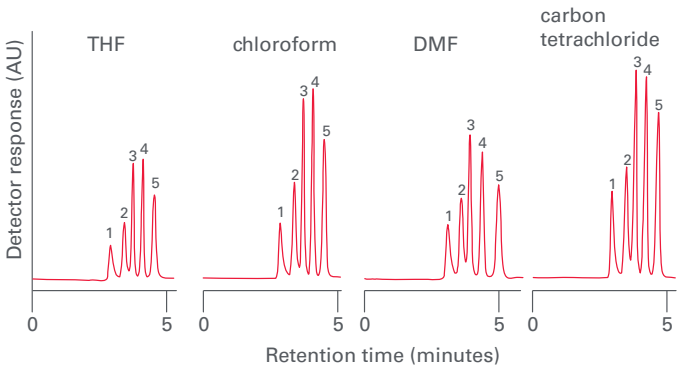
The following advantages are gained by conducting analysis at high temperature:

- Peaks become sharper as separation performance is increased. This is especially noticeable at higher flow rates.
- Viscosity of the mobile phase is lowered and operating pressure is decreased. This is an especially effective method with high-viscosity solvents such as DMSO, DMF, HFIP, etc.

Figure 59 demonstrates the temperature dependence of the separation of epoxy resin and a standard polystyrene mixture in TSKgel SuperH3000 and SuperH2500 columns.

FIGURE 58

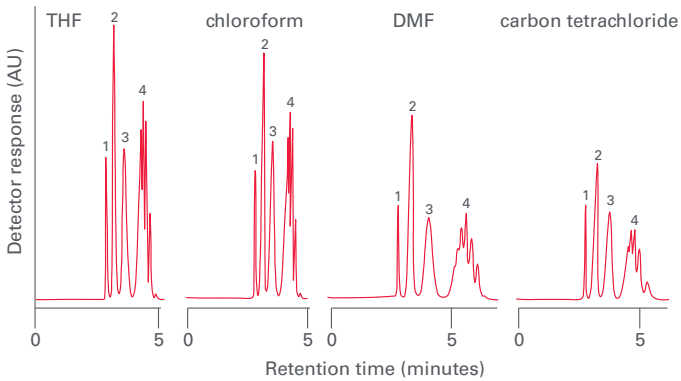
SEPARATION OF POLYSTYRENES ON TSKgel SuperHM-H



Column:	TSKgel SuperHM-H, 3 µm, 6 mm ID × 15 cm L
Mobile phase:	THF, chloroform, DMF, carbon tetrachloride
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV/VIS @ 254 nm
Sample:	1. polystyrene (2.89×10^6 Da)
	2. polystyrene (4.22×10^5 Da)
	3. polystyrene (1.07×10^5 Da)
	4. polystyrene (1.67×10^4 Da)
	5. polystyrene (2,800 Da)

FIGURE 57

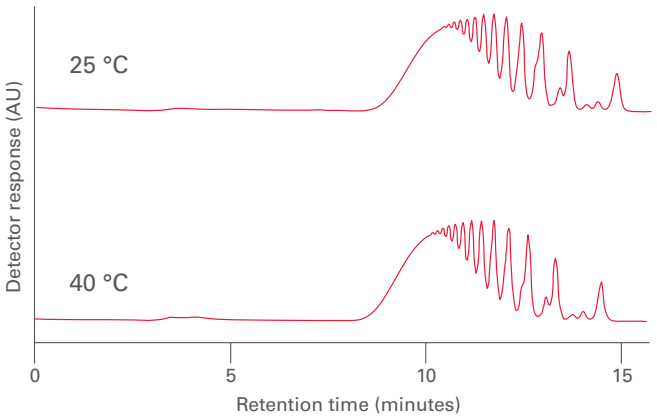
SEPARATION OF POLYSTYRENES TSKgel SuperH2500



Column:	TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm L
Mobile phase:	THF, chloroform, DMF, carbon tetrachloride
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV/VIS @ 254 nm or 270 nm
Samples:	1) polystyrene (1.9×10^5 Da)
	2) polystyrene (9.1×10^4 Da)
	3) polystyrene (2,800 Da)
	4) polystyrene A-500

FIGURE 59

TEMPERATURE DEPENDENCE OF SEPARATION ON EPOXY RESIN



Columns:	TSKgel SuperH3000, 3 µm, 6 mm ID × 15 cm L × 2
	TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm L × 3
Mobile phase:	THF
Flow rate:	0.6 mL/min
Detection:	UV @ 254 nm
Sample:	Epikote 1004 (0.1%), 10 µL

SEC/GPC
ABOUT TSKgel SuperHZ

The TSKgel SuperHZ column line consists of five columns of 4.6 mm ID and 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB, TSKgel SuperHZ1000 – 4000. Each column consists of a different pore size packing material. Subsequently, a unique separation range for each column exists, allowing researchers to choose a column that is designed for the sample type being analyzed.

The TSKgel SuperHZ column line also contains three mixed bed linear columns in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHZM-M to SuperHZM-N to SuperHZM-H. The mixed bed columns are also available in 4.6 mm ID and 6.0 mm ID × 15 cm.

TSKgel SuperHZ column dimensions are 6 mm ID × 15 cm and 4.6 mm ID × 15 cm versus 7.8 mm ID × 30 cm for conventional GPC columns. The smaller column dimensions translate to a reduction of peak volume by a factor of 3.4 (6 mm ID) and a factor of 5.8 (4.6 mm ID) versus the same component eluting from a corresponding TSKgel HXL column. Thus, your GPC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant.

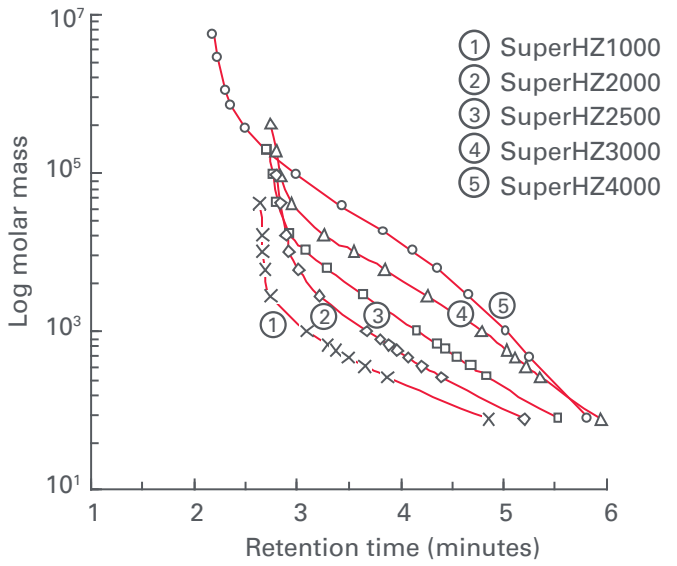
TSKgel SuperHZ columns have been developed for high throughput, high efficiency GPC applications such as those encountered in combinatorial chemistry experiments. These columns feature ultra-low sample adsorption.

TSKgel SuperHZ1000 – 4000 columns are capable of measuring monomers, polymer additives, oligomers and polymers up to a molar mass of several hundred thousand with proper selection of pore size. Ultra-fine particles (3 µm) have been developed to provide high resolution over the entire molar mass range. This is especially important for the separation of low molar mass compounds. Additionally, the mixed bed columns (TSKgel SuperHZM-N, M-M, and M-H) are capable of measuring oligomers and polymers with molar masses up to tens of millions with proper selection of the pore size. The various particle sizes of the mixed bed packing materials have been optimized to ensure resolution in the low molar mass range while avoiding shear degradation of polymers in the high molar mass region.

The columns are shipped in THF, which can be exchanged for a limited number of organic solvents as shown in Table VI. The calibration curves for the TSKgel SuperHZ columns are shown in Figures 60 and 61.

FIGURE 60

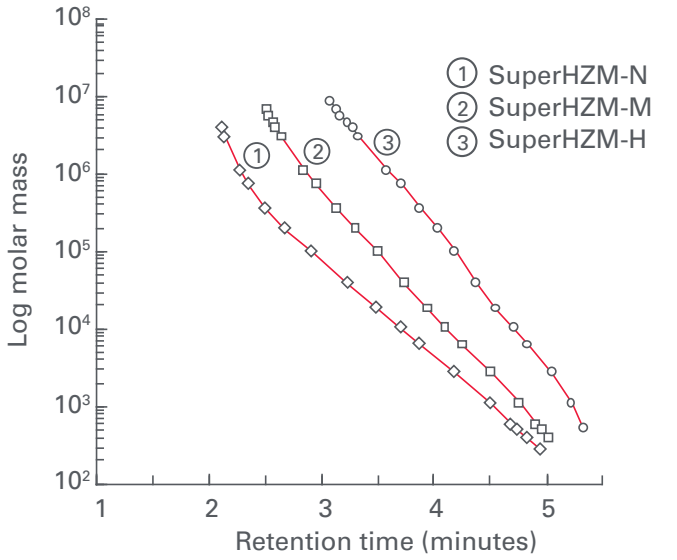
CALIBRATION CURVES FOR TSKgel SuperHZ COLUMNS



Column:	TSKgel SuperHZ columns, 4.6 mm ID × 15 cm L
Mobile phase:	THF
Flow rate:	0.35 mL/min
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	polystyrene standards

FIGURE 61

CALIBRATION CURVES FOR TSKgel SuperHZ MIXED BED COLUMNS



Column:	TSKgel SuperHZ columns, 4.6 mm ID × 15 cm L
Mobile phase:	THF
Flow rate:	0.35 mL/min
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	polystyrene standards

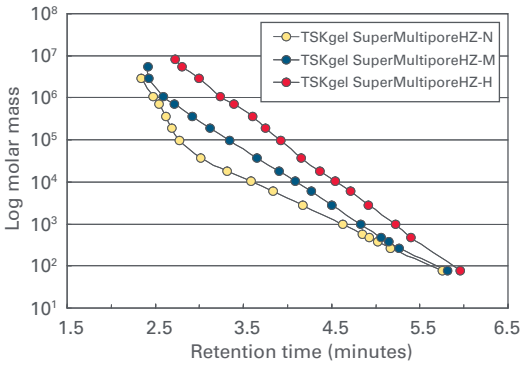
SEC/GPC
ABOUT TSKgel SuperMultiporeHZ

TSKgel SuperMultiporeHZ columns incorporate the multi-pore technology for the separation of polymers with a wide range of molar masses explained at the beginning of this chapter. The columns are packed with particles of a uniform size, with each particle having a very broad pore size distribution. This approach creates alinear calibration curve within each particle. The spherical monodisperse, 3, 4 or 6 µm particles consist of cross-linked polystyrene/divinylbenzene copolymer. This base material, coupled with the semi-micro column dimensions (4.6 mm ID × 15 cm), offers users high speed and low solvent consumption analyses with precise results. Three columns are available within the TSKgel SuperMultiporeHZ series, each with a different particle size and separation range: TSKgel SuperMultiporeHZ-N, SuperMultiporeHZ-M, SuperMultiporeHZ-H.

TSKgel SuperMultiporeHZ columns can be utilized for the analysis of polymers with a wide MM distribution range. The columns are shipped in THF, which cannot be replaced for any other organic solvent. Figure 62 shows the calibration curves for the TSKgel SuperMultiporeHZ columns.

FIGURE 62

CALIBRATION CURVES FOR TSKgel SuperMultiporeHZ COLUMNS



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

FEATURES

- Multi-pore packing material (wide range of pores contained in single particle)
- Smaller particle size (monodisperse particles)
- Semi-micro column
- Low adsorption packing material

BENEFITS

- Calibration curves with superior linearity
- No observable distortion of chromatograms
- mproved accuracy and repeatability of molar mass data
- Capable of rapid analysis with high separation performance
- Capable of achieving the same separation performance as conventional columns (30 cm) in half the analysis time
- No reduction in separation performance even for analysis at high flow rates
- mproved robustness of column performance
- Reduced solvent consumption
- 1/6th the consumption of conventional (30 cm) columns
- Can be used for a wide variety of samples

SEC/GPC
TSKgel SuperHZ APPLICATIONS

FAST ANALYSIS

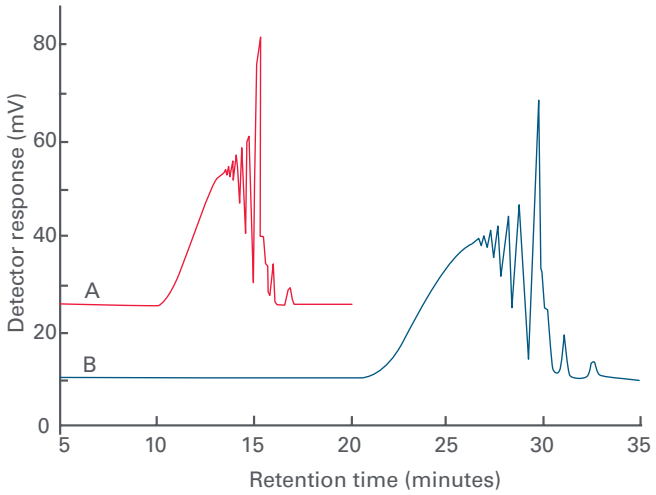
TSKgel SuperHZ1000-SuperHZ4000 columns are packed with 3 µm particles. The ultra-fine particles allow for high efficiency separations of low molar mass substances such as oligomers. These columns have theoretical plate values (per unit length) which are twice those of the conventional 5 µm columns. As a result, equal resolution can be obtained within half the analysis time. An example showing the analysis of phenolic resin is demonstrated in Figure 63.

VARIOUS POLYMERS

Various polymers were analyzed on four TSKgel SuperMultiporeHZ-M columns in series. The superimposed chromatograms in Figure 64 clearly demonstrate that these new GPC columns can be utilized for the analysis of polymers with a wide molar mass distribution range.

FIGURE 63

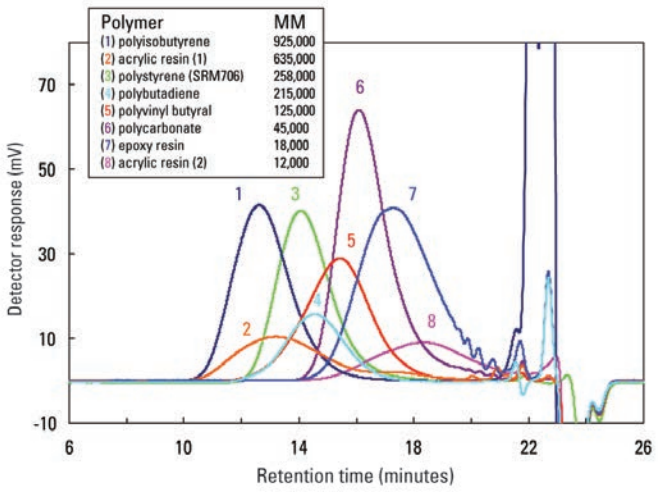
COMPARISON OF ANALYSIS ON TSKgel SuperHZ AND TSKgel HXL COLUMNS



Columns:	A. TSKgel SuperHZ columns (4000, 3000, 2500), 4.6 mm ID × 15 cm L × 3 B. TSKgel HXL columns (4000, 3000, 2500), 7.8 mm ID × 30 cm L × 3
Mobile phase:	THF
Flow rate:	A. 0.35 mL/min B. 1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	A. 5 µL B. 30 µL
Sample:	phenolic resin

FIGURE 64

SEPARATION OF VARIOUS POLYMERS



Columns:	SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm L × 4
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	10 µL
Sample conc.:	0.3%

SEC/GPC
TSKgel SuperHZ APPLICATIONS

STANDARD POLYSTYRENE

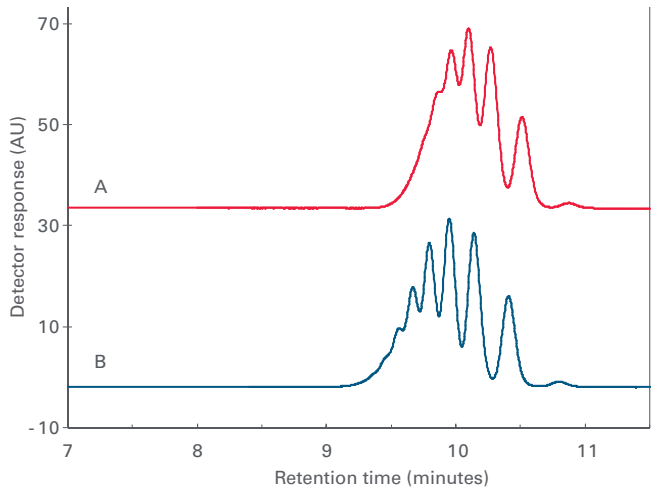
Figure 65 compares separation on the TSKgel SuperMultiporeHZ-N column versus the TSKgel SuperMultiporeHZ-M column in the low molar mass region (standard polystyrene A-500). The calibration curve for the TSKgel SuperMultiporeHZ-N column is not as steep and better separation is provided in the low molar mass region due to the smaller particle size (higher number of theoretical plates) of the TSKgel SuperMultiporeHZ-N column.

EPOXY RESIN

Figure 66 shows a chromatogram of an epoxy resin (approximately 6,000 Da) created using the TSKgel SuperMultiporeHZ columns. The best separation performance is shown by the TSKgel SuperMultiporeHZ-N column, the particle size used for low molar mass samples, and it is clear that the TSKgel SuperMultiporeHZ-H column does not provide adequate separation performance.

FIGURE 65

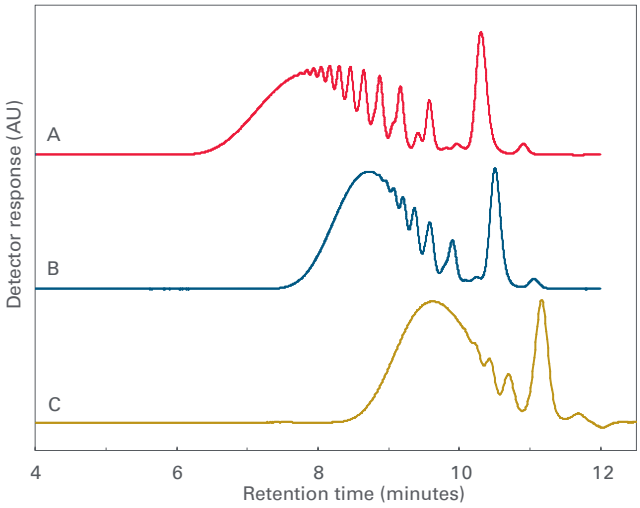
ANALYSIS OF STANDARD POLYSTYRENE



Columns:	A. TSKgel SuperMultiporeHZ-M, 4µm, 4.6 mm ID × 15 cm L × 2
	B. TSKgel SuperMultiporeHZ-N, 3µm, 4.6 mm ID × 15 cm L × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	5 µL
Sample:	standard polystyrene oligomer
	(TSKgel standard polystyrene A-500) (5 g/L)

FIGURE 66

ANALYSIS OF EPOXY RESIN



Columns:	A. TSKgel SuperMultiporeHZ-N, 3µm, 4.6 mm ID × 15 cm L × 2
	B. TSKgel SuperMultiporeHZ-M, 4µm, 4.6 mm ID × 15 cm L × 2
	C. TSKgel SuperMultiporeHZ-H, 6µm, 4.6 mm ID × 15 cm L × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	epoxy resin (3 g/L)

SEC/GPC
ORDERING INFORMATION TSKgel H SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel H Columns						
0017352	G1000H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016131	G1000H$_{XL}$	7.8	30	5	≥ 16,000	5.0
0017353	G2000H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016134	G2000H$_{XL}$	7.8	30	5	≥ 16,000	5.0
0017354	G2500H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016135	G2500H$_{XL}$	7.8	30	5	≥ 16,000	5.0
0017355	G3000H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016136	G3000H$_{XL}$	7.8	30	5	≥ 16,000	3.5
0017356	G4000H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016137	G4000H$_{XL}$	7.8	30	5	≥ 16,000	3.5
0017357	G5000H$_{HR}$	7.8	30	5	≥ 16,000	5.0
0016138	G5000H$_{XL}$	7.8	30	9	≥ 14,000	1.5
0017358	G6000H$_{HR}$	7.8	30	5	≥ 10,000	5.0
0016139	G6000H$_{XL}$	7.8	30	9	≥ 14,000	1.5
0017359	G7000H$_{HR}$	7.8	30	5	≥ 10,000	5.0
0016140	G7000H$_{XL}$	7.8	30	9	≥ 14,000	1.5
0017361	GMH$_{HR}$-H(S)	7.8	30	13		
0017362	GMH$_{HR}$-L mixed-bed	7.8	30	5	≥ 16,000	5.0
0018055	GMH$_{HR}$-N mixed-bed	7.8	30	5	≥ 16,000	5.0
0017392	GMH$_{HR}$-M mixed-bed	7.8	30	5	≥ 16,000	5.0
0017393	GMH$_{HR}$-M (S)	7.8	30	13	≥ 8,000	2.0
0018398	GMH$_{HR}$-H (30)	7.8	30	30	≥ 4,000	1.5
0018399	GMH$_{HR}$-H (20)	7.8	30	20	≥ 6,000	1.5
0017360	GMH$_{HR}$-H mixed-bed	7.8	30	5	≥ 16,000	5.0
0016652	GMH$_{XL}$-L mixed-bed	7.8	30	6	≥ 16,000	3.5
0016141	GMH$_{XL}$ mixed-bed	7.8	30	9	≥ 16,000	1.5
0017367	H$_{HR}$ (S) Guardcolumn	7.5	7.5		For GMH$_{HR}$ (S)	
0018402	H$_{HR}$ (30) Guardcolumn	7.5	7.5		For GMH$_{HR}$ (20) & (30)	
0017368	H$_{HR}$-L Guardcolumn	6.0	4.0	7	For G1000-4000H$_{HR}$ and GMH$_{HR}$-L columns	
0017369	H$_{HR}$-H Guardcolumn	6.0	4.0	7	For G5000-7000H$_{HR}$ and and GMH$_{HR}$-M; -N; -H columns	
0007113	H$_{XL}$-L Guardcolumn	6.0	4.0	7	For G1000H$_{XL}$ through G4000H$_{XL}$ columns	
0013727	H$_{XL}$-H Guardcolumn	6.0	4.0	13	For G5000H$_{XL}$ through GMH$_{XL}$-L mixed-bed columns	
H$_{HR}$ and H$_{XL}$ columns are packed in THF						
TSKgel Super H Columns						
0017990	SuperH1000	6.0	15	3	≥ 16,000	6.0
0017991	SuperH2000	6.0	15	3	≥ 16,000	6.0
0017992	SuperH2500	6.0	15	3	≥ 16,000	6.0
0017993	SuperH3000	6.0	15	3	≥ 16,000	4.0
0017994	SuperH4000	6.0	15	3	≥ 16,000	4.0

SEC/GPC
ORDERING INFORMATION TSKgel H SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
0017995	SuperH5000	6.0	15	3	≥ 16,000	4.0
0017996	SuperH6000	6.0	15	5	≥ 16,000	4.0
0017997	SuperH7000	6.0	15	5	≥ 16,000	4.0
0017998	SuperHM-L	6.0	15	3	≥ 16,000	4.0
0017999	SuperHM-N	6.0	15	3	≥ 16,000	4.0
0018000	SuperHM-M	6.0	15	3	≥ 16,000	4.0
0018001	SuperHM-H	6.0	15	3 and 5	≥ 16,000	4.0
0019302	TSKgel SuperHZ1000	6.0	15	3	≥ 16,000	5.6
0019303	TSKgel SuperHZ2000	6.0	15	3	≥ 16,000	5.0
0019304	TSKgel SuperHZ2500	6.0	15	3	≥ 16,000	4.0
0019305	TSKgel SuperHZ3000	6.0	15	3	≥ 16,000	3.0
0019306	TSKgel SuperHZ4000	6.0	15	3	≥ 16,000	3.5
0019309	TSKgel SuperHZ1000	4.6	15	3	≥ 16,000	5.6
0019310	TSKgel SuperHZ2000	4.6	15	3	≥ 16,000	5.0
0019311	TSKgel SuperHZ2500	4.6	15	3	≥ 16,000	4.0
0019312	TSKgel SuperHZ3000	4.6	15	3	≥ 16,000	3.0
0019313	TSKgel SuperHZ4000	4.6	15	3	≥ 16,000	3.5
0019660	TSKgel SuperHZM-N	4.6	15	3	≥ 16,000	3.5
0019661	TSKgel SuperHZM-N	6.0	15	3	≥ 16,000	3.5
0019662	TSKgel SuperHZM-M	4.6	15	3 and 5	≥ 16,000	2.0
0019663	TSKgel SuperHZM-M	6.0	15	3 and 5	≥ 16,000	2.0
0019664	TSKgel SuperHZM-H	4.6	15	10	≥ 9,000	1.0
0019665	TSKgel SuperHZM-H	6.0	15	10	≥ 9,000	1.0
Guardcolumns						
0018002	SuperH-L Guardcolumn	4.6	3.5	3	For SuperH1000-4000	
0018003	SuperH-H Guardcolumn	4.6	3.5	3	For SuperH5000-7000 and HM-L;-N;-M;-H columns	
0019666	SuperHZ-L Guardcolumn	4.6	3.5	4	For 6.0 mm ID SuperHZ1000-4000 and HZM-N &-M columns	
0019667	SuperHZ-H Guardcolumn	4.6	3.5	10	For 6.0 mm ID SuperHZM-H columns	
0019668	SuperHZ-H Guardcolumn	4.6	2.0	10	For 4.6 mm ID SuperHZM-H columns	
0019314	SuperHZ-L Guardcolumn	4.6	2.0	4	For 4.6 mm ID SuperHZ1000-4000 and HZM-N &-M	
TSKgel Multipore Columns						
0018403	Multipore HXL-M	7.8	30	5	≥ 16,000	3.5
0018404	MultiporeH-M Guardcolumn	6.0	4.0	5	For P/N 0018403	
0021488	SuperMultiporeHZ-M	4.6	15	4	≥ 16,000	2.4
0021489	SuperMultipore-M Guardcolumn	4.6	2.0	4	For SuperMultipore HZ-M P/N 0021488	
0021815	SuperMultiporeHZ-N	4.6	15	3	≥ 20,000	4.0
0021816	SuperMultipore-N Guardcolumn	4.6	2.0	3	For SuperMultipore HZ-N P/N 0021815	
0021885	SuperMultiporeHZ-H	4.6	15	6	≥ 11,000	1.0
0021886	SuperMultiporeH Guardcolumn	4.6	2.0	6	For SuperMultipore HZ-H P/N 0021887	

SEC/GPC
ORDERING INFORMATION TSKgel H SERIES

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel GPC columns for high temperature GPC						
0022887	GMH$_{HR}$-H (30) HT2**	7.8	30		For HT-GPC up to 220 °C	
0022888	GMH$_{HR}$-H (20) HT2**	7.8	30		For HT-GPC up to 220 °C	
0022889	GMH$_{HR}$-H (S) HT2**	7.8	30		For HT-GPC up to 220 °C	
0022890	G2000H$_{HR}$ (20) HT2**	7.8	30		For HT-GPC up to 220 °C	
0018391	GMH$_{HR}$-H(30)HT mixed-bed	7.8	30	30	≥ 4,000	1.5
0018392	GMH$_{HR}$-H(20)HT mixed-bed	7.8	30	20	≥ 6,000	1.5
0018393	GMH$_{HR}$-H(S)HT mixed-bed	7.8	30	13	≥ 8,000	2.0
Guardcolumns for High Temperature GPC						
0022891	H$_{HR}$ (30) HT2** Guardcolumn	7.5	7.5		For HT-GPC up to 220 °C	
0022892	H$_{HR}$ (S) HT2** Guardcolumn	7.5	7.5		For HT-GPC up to 220 °C	
0018397	GMH$_{HR}$-H (S)HT* Guardcolumn	7.5	7.5		For HT-GPC up to 170 °C	

H$_{HR}$-HT/HT2 and H$_{XL}$-HT/HT2 columns are packed in ODCB, HT Temp. max 170 °C; HT2** Temp. max 220 °C*

Columns for reference flow line of EcoSEC GPC Systems					
0018004	SuperH-RC Reference column	6.0	15	4	Reference column for EcoSEC
0022893	H$_{HR}$ HT-RC Reference Column	7.5	7.5		Reference Column for EcoSEC HT

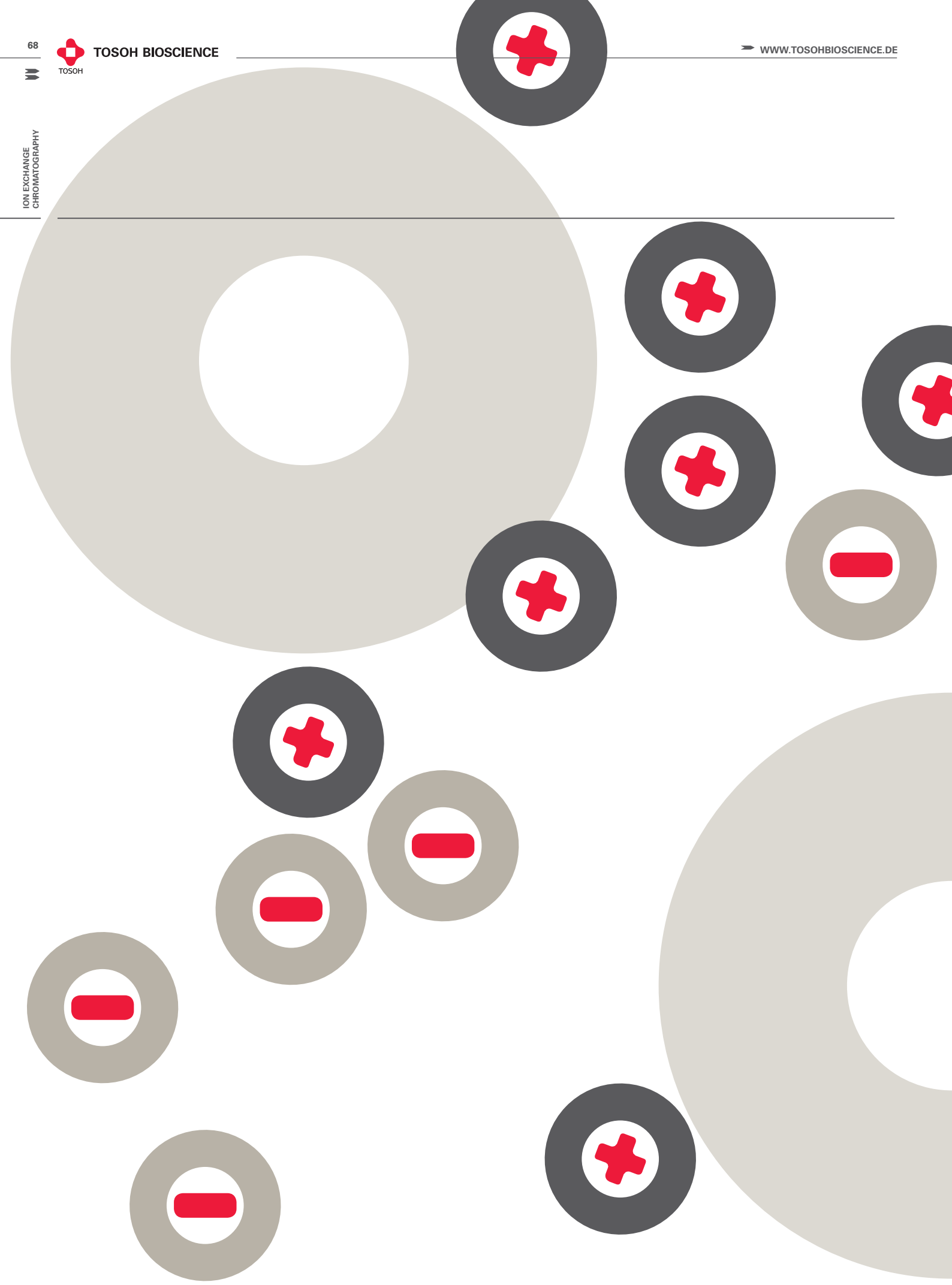
Part #	Description	Nominal MW (Da)	Amount
TSKgel polymer standards			
Polystyrene			
To calibrate TSKgel SuperMultiporeHZ columns			
0021912	PStQuick MP-N	5.3 x 10^2 - 4.4 x 10^4	60 vials
0021913	PStQuick MP-M	5.3 x 10^2 - 8.0 x 10^5	60 vials
0021914	PStQuick MP-H	9.5 x 10^2 - 5.5 x 10^6	60 vials
To calibrate TSKgel H-type mixed-bed columns			
0021915	PStQuick Kit-L	5.3 x 10^2 - 4.2 x 10^5	40 vials
0021916	PStQuick Kit-M	5.3 x 10^2 - 2.9 x 10^6	40 vials
0021917	PStQuick Kit-H	5.3 x 10^2 - 8.4 x 10^6	60 vials
To calibrate standard TSKgel GPC columns			
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

SEC/GPC
ORDERING INFORMATION POLYMER STANDARDS

ORDERING INFORMATION

Part #	Description	Nominal MW (Da)	Amount
TSKgel polymer standards:			
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	1.260.000 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-128		12 x 1 g
0006477	High MW Kit, F-10 thru F-2000		12 x 1 g
Polyethylene oxide			
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 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.

IEC HIGHLIGHTS

HIGHLIGHTS TSKgel STAT SERIES

- TSKgel STAT columns provide high efficiency separations at short analysis time
- Very efficient separation for high as well as low MW solutes through novel bonding chemistry and the absence of pores
- High speed and high resolution analysis of biomolecules in HPLC and UHPLC systems
- Higher adsorption capacities and lower pressures compared with smaller particle sized TSKgel NPR columns

HIGHLIGHTS TSKgel BioAssist COLUMNS

- Pore structure and bonding chemistry of provide high capacity for small to very large MW proteins and nucleic acids
- Suitable for use in systems that are designed for HPLC, laboratory or semipreparative applications
- TSKgel BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware.

FEATURES

- Polymer- and silica-based stationary phases
- Selection of strong and week ion exchange ligands
- Broad range of pore sizes available
- Non-porous base particles available
- Bioinert column hardware available

BENEFITS

- Select ideal matrix hydrophobicity and pH stability
- Find the perfect selectivity for any application
- Select a column that fits to the molecular weight of your sample
- deal for fast analysis, e.g. in QC or process monitoring
- Less sample loss through adsorption

IEC
HOW DOES IT WORK?

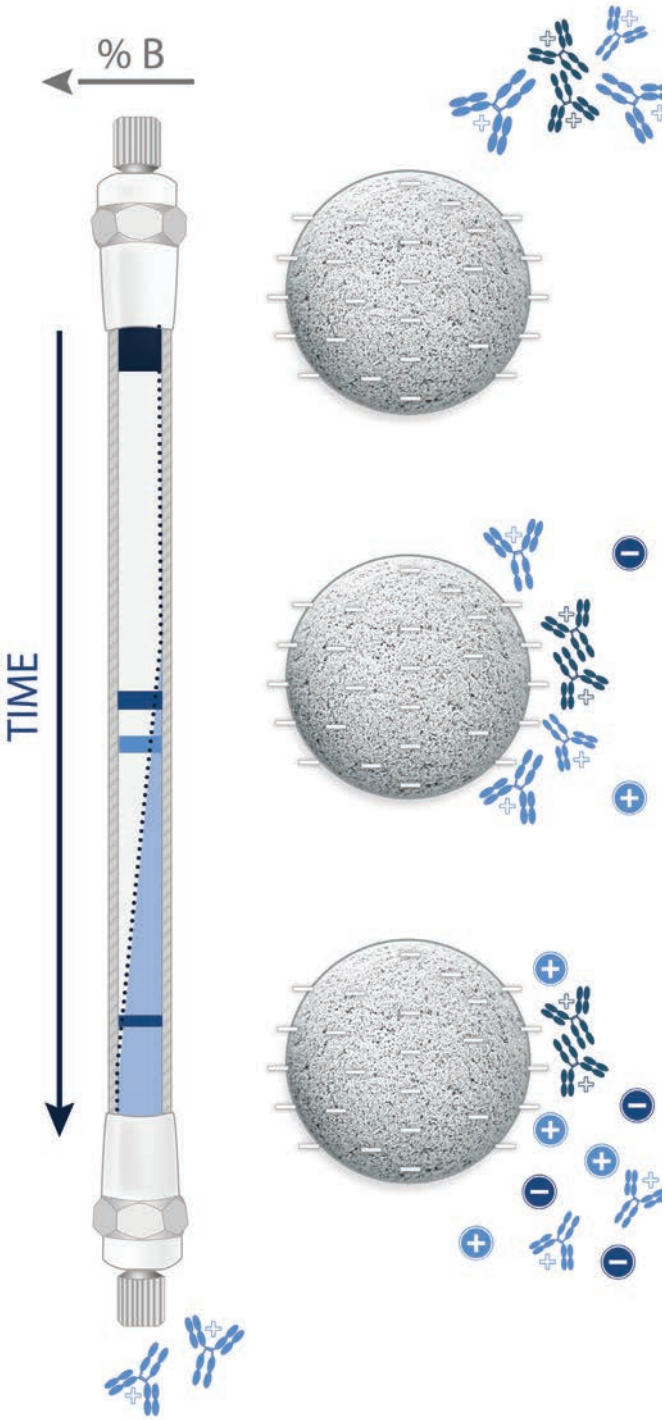
Ion Exchange Chromatography (IEC) retains molecules based on ionic interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge. IEC is further subdivided into cation exchange and anion exchange chromatography: anion exchange phases carry positively charged groups that attract negatively charged molecules; cation exchange resins display negatively charged groups which attract positively charged molecules. Charged target molecules are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged ion that will displace the analyte ions from the stationary phase or by applying a pH gradient changing the overall charge of the analyte.

Proteins have numerous functional groups that can have both positive and negative charges. IEC separates proteins according to their net surface charge, which is dependent on the pH and ionic strength of the mobile phase. According to differences in their overall charge and surface charge distribution, proteins can be separated by IEC. IEC takes advantage of the fact that the relationship between net surface charge and pH is unique for a specific protein. At a pH, equivalent to its isoelectric point, a protein has no net charge and will not interact with the charged stationary phase. At a pH above the pI the protein will have a negative net charge and will therefore bind to a positively charged anion exchanger. At a pH below its pI it will have a positive net charge and will consequently interact with a negatively charged cation exchanger. By adjusting the pH or the salt concentration of the mobile phase, separation can be optimized.

For loading, the pH and ionic strength are selected in a way that the analytes bind to the stationary phase (Figure 1). Elution is usually performed by changing the ionic strength of the mobile phase by applying a salt gradient. As the salt concentration of the mobile phase increases, the salt ions compete with the bound molecules for the functional groups of the stationary phase. The higher the net charge of the molecule, the higher the salt concentration needed for elution. Very tightly bound compounds are removed at the end of the elution by a wash step with very high salt buffer.

FIGURE 1

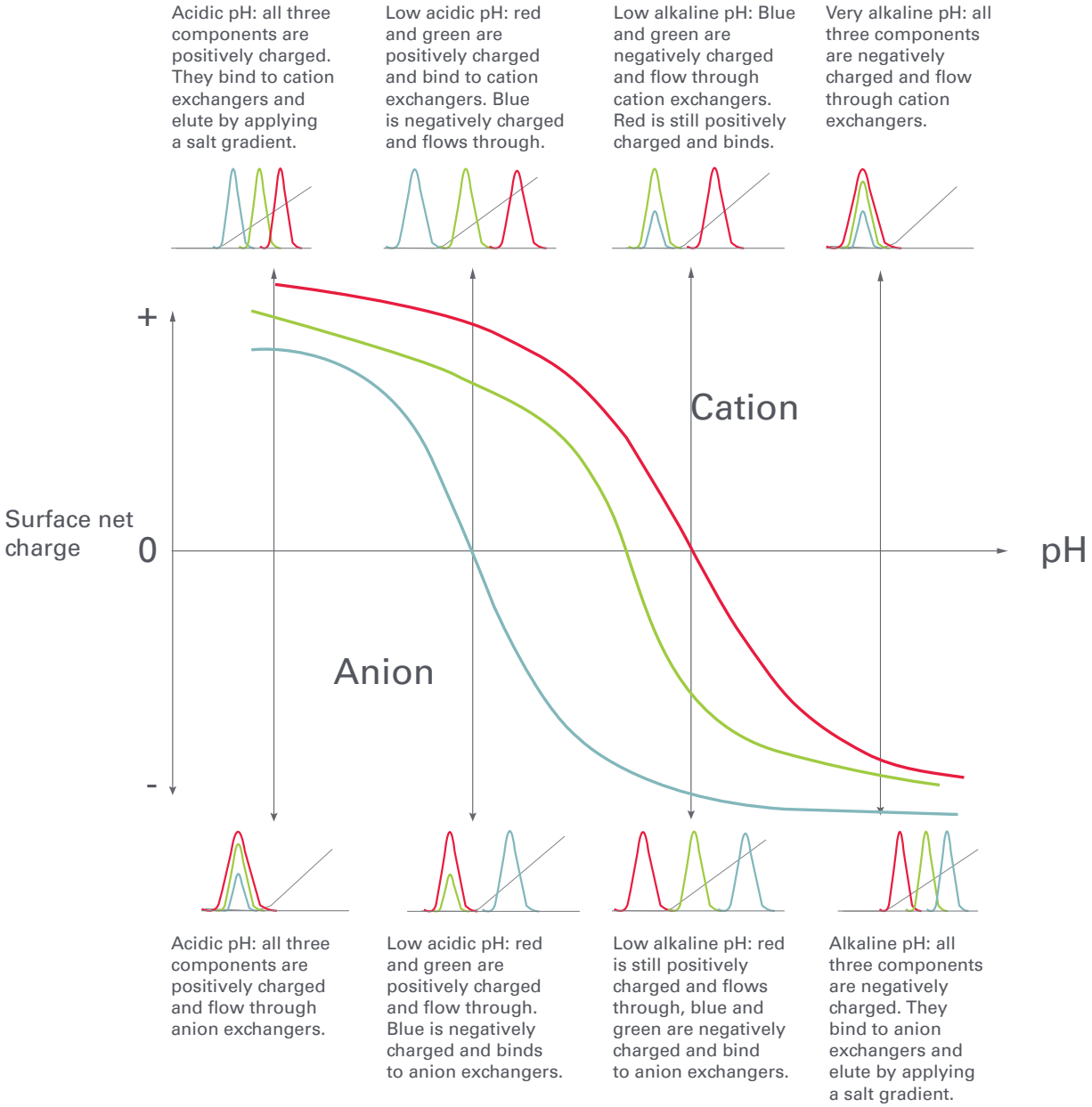
ION EXCHANGE CHROMATOGRAPHY ILLUSTRATION



IEC
HOW DOES IT WORK?

FIGURE 2

BINDING TO ION EXCHANGE GROUPS



IEC
STATIONARY PHASES

Ion exchange stationary phases are classified as weak or strong ion exchangers. The terms strong and weak do not refer to the performance of the stationary phase or to the strength of interaction between particle and target. 'Strong', respectively 'weak' refers to the extent that the ion exchange capacity varies with change in pH. Strong ion exchange groups have a steep titration curve. They show no variation of their ionization state with the pH and remain fully charged over a broad pH range.

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by anion and cation exchange chromatography. Most of the available chemistries are offered in analytical as well as semi-preparative formats. Particle sizes range from 2.5 µm for fast analysis to 13 µm for preparative purposes. proteins, peptides, oligonucleotides, and nucleic acids are typical samples that are analyzed or isolated by IEC.

PACKING MATERIALS AND CHEMISTRIES

Polymethacrylate, silica and polystyrene are used as matrices for the TSKgel line of ion exchange columns.

The base resins are derivatized either with diethylaminoethyl (DEAE), quaternary ammonium (Q), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide weak anion, strong anion, strong cation and weak cation exchangers, respectively.

The polymethacrylate backbone of TSKgel STAT, NPR, and PW-type columns provides a robust, hydrophilic particle suitable as a support for high performance separations of biomolecules. The advanced non-porous resin column technology featured in the TSKgel NPR and STAT series eliminates rate-limiting pore diffusion. Thus, analysis time is often reduced by as much as 80% without loss in resolution and recoveries are routinely greater than 90%. In addition, the innovative bonding chemistry of TSKgel STAT series results in columns that show a reasonable higher sample capacity than traditional non-porous resins.

The silica-based TSKgel SW series is typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides or small peptides. For special applications polystyrene-based columns are offered. E. g. for analyzing small molecular weight sugars, amino acids, nucleic acids or small drug candidates.

FEATURES	BENEFITS
Polymer-Based Ion Exchange Columns	
Methacrylate backbone	Mechanically and chemically stable (pH 2.0-12.0) withstands repeated alkaline cleaning and use of organic solvents, denaturants and surfactants
Non-porous particle based (STAT and NPR) columns	Fast QC analysis and process monitoring
Large pore size (100 nm) (excl. limit for proteins ~ 5,000,000 Da)	Use same column for most biopolymers
BioAssist Columns	
Polymer matrix with large pores	High capacity even for larger proteins (1 million Da)
Unique pore structure providing 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
Silica-Based Ion Exchange Columns	
Smaller pore size (2SW = 12.5 nm and 3SW = 25 nm)	Most suitable for analyzing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins

IEC COLUMN SELECTION

TABLE I

TSKgel ION EXCHANGE COLUMN SELECTION

Sample type	MW range (Da)	TSKgel column	pH range
Amino acids, peptides and proteins			
Amino acids	< 2,000	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

IEC
COLUMN SELECTION

WHICH ION EXCHANGE COLUMN SHOULD I EVALUATE?

- Top-performer for fast, highly efficient biomolecule separation – TSKgel STAT
- First choice for very large proteins – TSKgel BioAssist
- Small molecular weight molecules – TSKgel 2SW/3SW

IEC
ABOUT TSKgel ANION EXCHANGE COLUMNS

- TSKgel Q- and DNA-STAT columns provide high efficiency separations at short analysis time
- TSKgel DNA-NPR columns are ideal for PCR fragment analysis
- Pore structure and bonding chemistry of TSKgel BioAssist Q columns provide high capacity for small to very large MW proteins and nucleic acids
- Specialty columns for analysis of mono- and disaccharides and sugar alcohols are available

TSKgel STAT ANION EXCHANGE COLUMNS

These are non-porous polymer columns with high surface density of quaternary ammonium functional groups (Q- and DNA-STAT). Particle sizes and dimensions are optimized either for highest throughput or for highest efficiency. Applications for the TSKgel STAT columns include the separation of proteins, DNA fragments, nucleic acids, oligo DNA, and siRNA.

TSKgel DEAE-5PW AND SuperQ-5PW

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with diethylaminoethyl (DEAE) to provide a weak anion exchanger. The polyamine chemistry employed in TSKgel SuperQ-5PW results in a high capacity strong anion exchanger with a smaller effective pore size than TSKgel DEAE-5PW. Proteins, peptides, DNA- and RNA-derived oligonucleotides, and other nucleic acid fragments are typical samples that are separated on the polymer-based TSKgel ion exchange columns.

TSKgel BioAssist ANION EXCHANGE COLUMNS

These columns are also based on methacrylate particle design technology. Particles in TSKgel BioAssist Q columns contain very large pores (~400 nm) that are functionalized with polyamine groups to form a network structure. The capacity of the TSKgel BioAssist Q columns is high over a wide molecular weight range (up to 1.0×10^6 Da). TSKgel BioAssist columns are available exclusively in PEEK housing.

TSKgel DEAE-NPR AND DNA-NPR

TSKgel DEAE-NPR and DNA-NPR anion exchange columns Polymethacrylate is the backbone of these non-porous resin columns, which 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. Due to the absence of large pores, protein recovery is generally very high on TSKgel NPR columns.

TSKgel DEAE-2SW AND DEAE-3SW

Silica-based TSKgel anion exchange columns with diethylaminoethyl (DEAE) functional groups are available for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins. Binding capacity for small to medium size proteins on these columns is approximately double that of the TSKgel 5PW packings due to the smaller pore size and larger surface area.

SPECIALTY TSKgel ANION EXCHANGE COLUMNS

These columns are available for the analysis of mono and disaccharides and sugar alcohols.

IEC
ABOUT TSKgel ANION EXCHANGE COLUMNS

TABLE II

FEATURES AND BENEFITS OF TSKgel ANION EXCHANGE COLUMNS

TSKgel Column Type	Type/Matrix	Benefit
Q-STAT, DNA-STAT	strong (Q-STAT), strong (DNA-STAT), polymer	Non-porous with high surface density of quaternary ammonium groups
DEAE-5PW, SuperQ-5PW	strong (SuperQ-5PW), weak (DEAE-5PW)/polymethacrylate	Polymethacrylate resin derivatized with diethylamino-ethyl (DEAE) and trimethylamino (SuperQ) ligands
BioAssist Q	strong/polymethacrylate	Contain very large pores (400 nm), resulting in high binding capacity and improved recovery of activity; available exclusively in PEEK housing
DEAE-NPR, DNA-NPR	weak/polymethacrylate	Non-porous with 2.5 µm particles; fast analysis; high protein recovery
DEAE-2SW, DEAE-3SW, QAE-2SW	strong (QAE-2SW), weak (DEAE-2SW, DEAE-3SW)/silica	Silica-based with diethylaminoethyl (DEAE), and trime-thylamino (QAE) functional groups
Sugar AXG, Sugar AXI, SAX	strong/polystyrene	Specialty columns for the analysis of mono and disac-charides, as well as organic acids and sugar alcohols

TABLE III

PROPERTIES OF TSKgel ION EXCHANGE COLUMNS

TSKgel	Ma-trix*	Particle size (µm)	Pore size (nm)	Func-tional group	Counter ion	Excl. limit PEG** (Da)	Capac-ity (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

** pMA = poly methacrylate; PS-DVB = polystyrene-divinylbenzene*
*** Polyethylene glycol*
**** PEEK = polyethyletherketone, S = stainless steel, G = glass*

IEC
ABOUT TSKgel Q-/DNA-STAT

TSKgel Q-STAT and DNA-STAT columns are packed with hydrophilic non-porous resin particles of which the surface consists of an open access network of multi-layered anion exchange groups (see Figure 3). The innovative bonding chemistry results in a respectable loading capacity.

TSKgel STAT anion exchange columns are available in various column formats and particle sizes to perfectly match specific application needs. For fast and ultra-fast analysis 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.

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 summarized in Table IV.

FIGURE 3

SCHEMATIC DIAGRAM OF TSKgel STAT SERIES

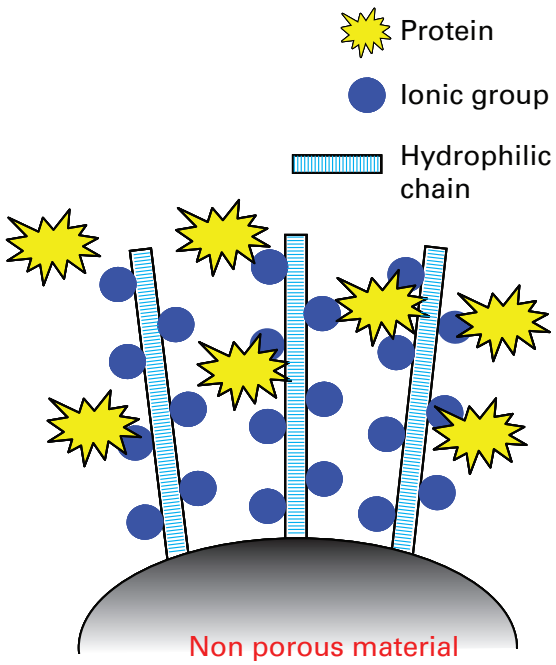


TABLE IV

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

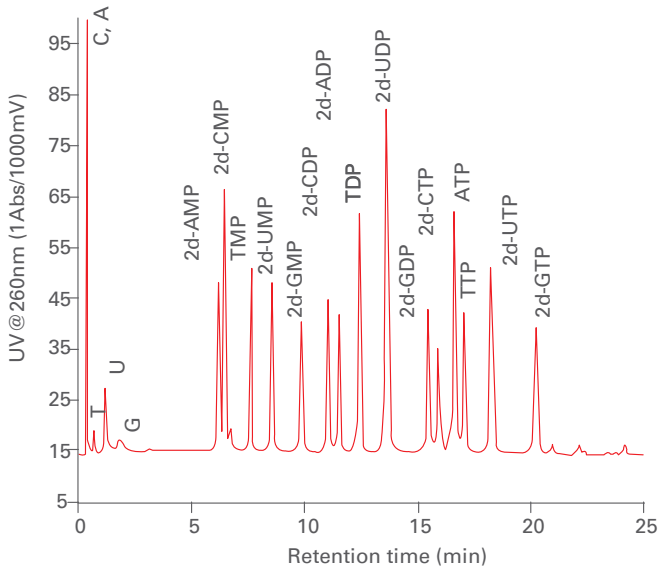
IEC
TSKgel Q-/DNA-STAT APPLICATIONS

ANALYSIS OF NUCLEOTIDES

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column packed with 5µm particles. The narrow, symmetrical peaks, as shown in Figure 4, demonstrate the absence of micropores on this new generation of non-porous resin columns.

FIGURE 4

HIGH RESOLUTION SEPARATIONS OF NUCELOTIDES



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

IEC
ABOUT TSKgel DNA-/ DEAE-NPR

TSKgel DNA-NPR columns are packed with 2.5 µm hydrophilic non-porous polymer beads which are modified with a weak anion exchange group. Column dimensions are optimized for the high efficiency separation of DNA fragments, PCR products, or plasmids. Binding capacity of non-porous particles is low compared to porous particles with the same ligand functionality but resolution is higher.

The hydrophilic polymer beads used to pack the TSKgel DEAE-NPR columns are also modified with a weak anion exchange group. These columns are used for the high speed separation of proteins, oligo- and polynucleotides. TSKgel DEAE-NPR columns are particularly useful for high resolution separation of DNA digests or fragments.

TSKgel DNA-NPR APPLICATIONS

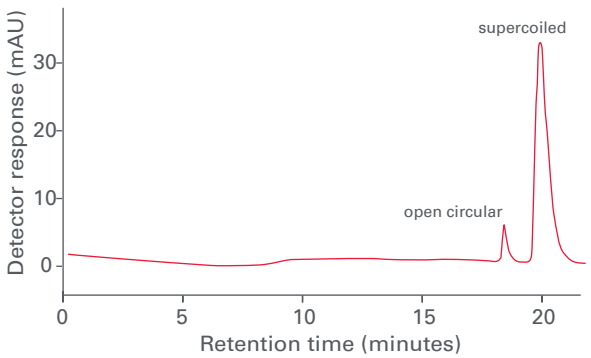
Plasmid Analysis
One of the purity checks used for plasmids is the measure of the relative amount of open circular plasmid versus supercoiled plasmid. Figure 5 demonstrates the utility of the TSKgel DNA-NPR column for this type of analysis.

QC Analysis of Oligonucleotides
Figure 6 shows the chromatographic trace of the crude deprotected 13-mer oligonucleotide using a TSKgel DNA-NPR column. The early eluting peaks from 0 – 5 minute exhibit a lambda max range of 220–230 nm, indicating the presence of protecting groups used in the synthesis. The N-1 peak as confirmed by mass spectrometry elutes just before the main substance peak. The PS=PO peak elutes before N-1. Structurally, the N-1 analog is completely thioated but is missing one nucleotide. As a result, the N-1 compound is more thioated and hydrophobic than the PS=PO analog. The backside peak is an N+1 impurity verified by mass spectrometry.

The method conditions are designed to optimize resolution of all impurity peaks and inhibit any aggregation, secondary structure formation, and PS=PO conversion. Specifically, sodium bromide acts as the eluting agent and diethylamine provides the buffering capacity while contributing mild chaotropic effects. The step gradient is designed to remove all the protecting groups from the column before elution of the impurity analogs.

FIGURE 5

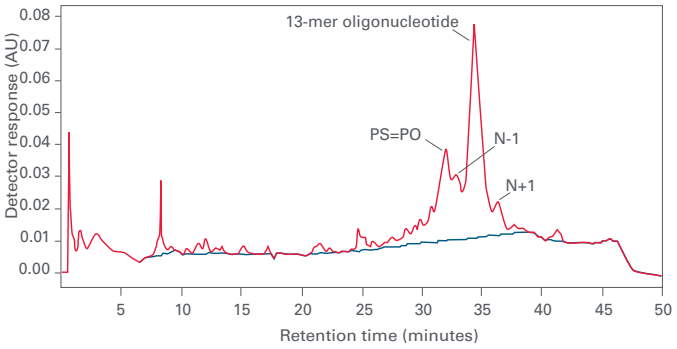
PLASMID ANALYSIS



Column:	TSKgel DNA-NPR, 4.6 mm ID x 7.5 cm L
Mobile phase:	A. 20 mmol/L Tris, pH 9.0: B. 20 mmol/L Tris, pH 9.0 with 1 mol/L NaCl linear gradient from 50% to 65% B in 10 column volumes
Flow rate:	1 mL/min
Detection:	UV @ 260nm
Samples:	PUC19 plasmid

FIGURE 6

OLIGONUCLEOTIDE ANALYSIS



Column:	TSKgel DNA-NPR, 2.5 µm, 4.6 mm ID × 7.5 cm L
Mobile phase:	A: 10 mmol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine B: 1 mol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine
Gradient	3.5 min (20%B) 12 min (20%B) 45 min (55%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 260nm
Temperatures:	60 °C (column), 4 °C (sample chamber)
Sample:	crude deprotected 13-mer oligonucleotide

IEC
TSKgel DEAE-NPR APPLICATIONS

Analysis of DNA digests

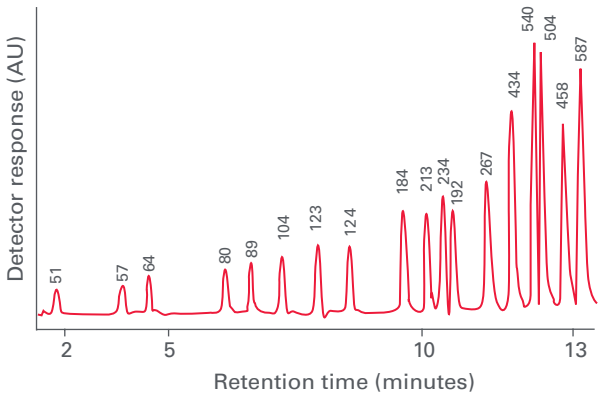
Because of their small particle size, TSKgel DEAE-NPR non-porous columns excel in rapid separations of large polynucleotides in DNA digests. A chromatogram of a standard Hae III digest of pBR322 plasmid DNA is shown in Figure 7.

HIV-1 PCR-amplified 130 bp Target

Figure 8 shows the detection of a 130 bp target derived from HIV using a non-porous TSKgel DEAE-NPR column.

FIGURE 7

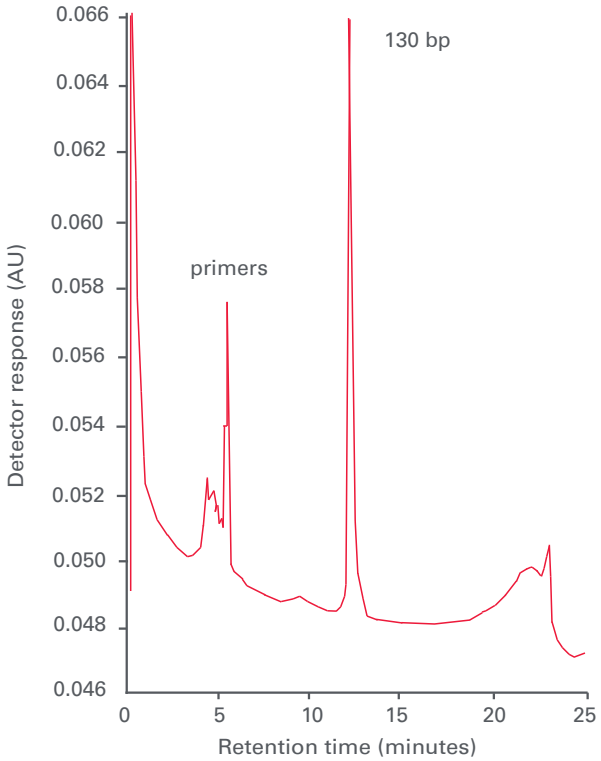
ANALYSIS OF DNA DIGEST



Column:	TSKgel DEAE-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm L with guard column, 4.6 mm ID × 0.5 cm L
Mobile phase:	A: 0.02 mol/L Tris-HCl, pH 9.0 B: mobile phase A plus 1.0 mol/L NaCl
Gradient:	15 min linear gradient from 48% to 65% mobile phase B
Flow rate:	1.5 mL/min
Detection:	UV @ 260 nm
Pressure:	14 MPa
Temperature:	40 °C
Sample:	Hae III digest of pBR322 DNA, (base pair number for each peak is indicated)

FIGURE 8

DETECTION OF HIV-1 PCR-AMPLIFIED 130 bp TARGET



Column:	TSKgel DEAE-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm L
Mobile phase:	A: 20 mmol/L Tris-HCl with 0.25 mol/L NaCl, pH 7.7 B: 20 mmol/L Tris-HCl with 1 mol/L NaCl, pH 7.7
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Temperature:	ambient
Sample:	HIV-1 PCR-amplified 130 bp target
Sample load:	20 µL

IEC
ABOUT TSKgel BIOASSIST Q

Especially designed for the separation of large biomolecules, such as antibodies, the large pores of the TSKgel BioAssist Q column offer superior capacity and resolution at a modest back pressure. The anion exchange functionality of BioAssist Q columns is introduced via a special graft polymerization technique that results in a high density of ionic exchange groups in the large particle pores that normally could only be achieved by using particles containing a much smaller pore size.

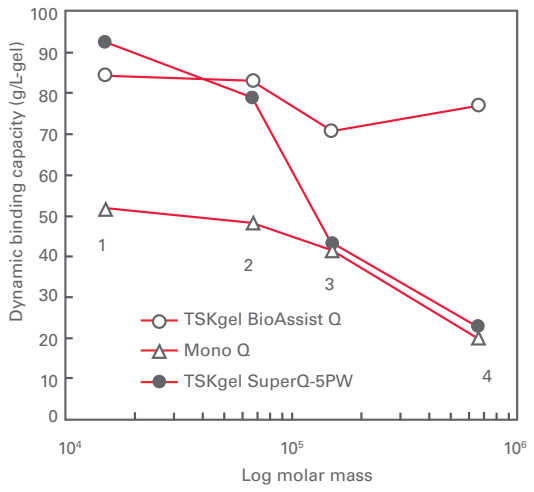
TSKgel BioAssist Q columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale-up. The hardware for both columns is made of PEEK.

DYNAMIC BINDING CAPACITY

The dynamic binding capacity for a TSKgel BioAssist Q column and two commercially available columns is shown in Figure 9 as a function of protein molar mass. Dynamic capacity is plotted against the molar mass of 4 proteins varying in molar mass from 2.0 × 10^4 Da to 6.7 × 10^5 Da and is determined by continuously loading the column with the protein solution and calculating the amount of protein adsorbed at 10% height of the breakthrough curve.

FIGURE 9

DYNAMIC BINDING CAPACITY AS FUNCTION OF PROTEIN SIZE



Columns: TSKgel BioAssist Q, 10 µm, 4.6 mm ID × 1 cm L
Conventional Q-type product A, 5.0 mm ID × 1 cm L
TSKgel SuperQ-5PW, 4.6 mm ID × 1 cm L
Mobile phase: 20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate: 0.38 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Samples: 1. trypsin inhibitor, 10 g/L
2. human serum albumin, 10 g/L
3. IgG_1, 2.3 g/L
4. thyroglobulin, 5 g/L

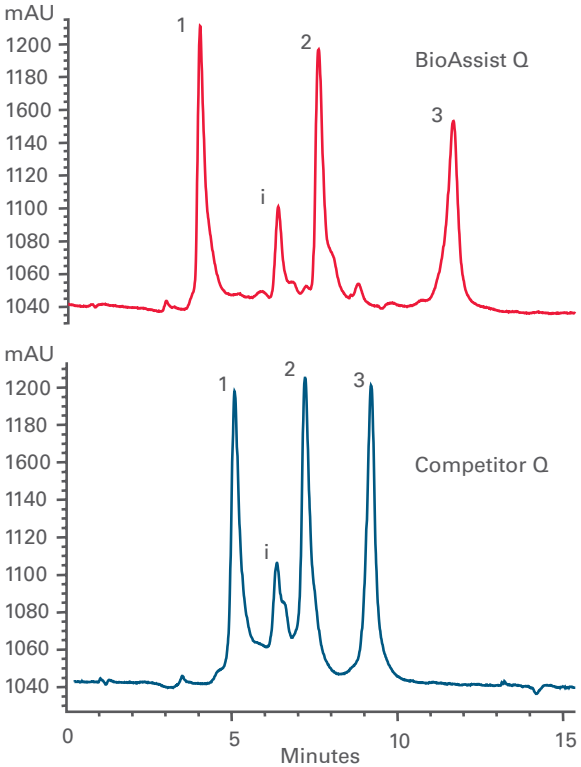
The binding capacity on TSKgel BioAssist Q is uniformly high for all proteins, while that of Mono Q (80 nm pores) and TSKgel SuperQ-5PW (100 nm pores) is distinctly lower for the larger proteins. It is evident that neither material is optimized for the analysis of monoclonal antibodies, which have a molar mass of 1.5 × 10^5 Da.

TSKgel BioAssist Q APPLICATIONS

The polymer based TSKgel BioAssist Q column with large pores is suitable for use in systems that are designed for laboratory or semi-preparative applications. Figure 10 demonstrates the performance enhancement of TSKgel BioAssist Q over a competitive product when operated side-by-side on an FPLC system.

FIGURE 10

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
Mobile phase: 30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate pH 8.0
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Sample: 1) conalbumin, i) ovalbumin impurity,
2) ovalbumin
3) trypsin inhibitor

IEC
ABOUT TSKgel DEAE-/SUPERQ-5PW

The polymer-based TSKgel 5PW is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with a diethylaminoethyl (DEAE) functionality to provide the weak anion exchange column TSKgel DEAE-5PW, and with a polyamine functionality to provide the strong anion exchange column TSKgel SuperQ-5PW.

The polyamine network chemistry employed in TSKgel SuperQ-5PW results in a much higher capacity, but also a smaller effective pore size than TSKgel DEAE-5PW.

COLUMN STABILITY

Figures 11A & 11B demonstrate the stability of TSKgel SuperQ-5PW. Ovalbumin and trypsin inhibitor were initially loaded onto a TSKgel SuperQ-5PW, 7.5 mm ID × 7.5 cm column (Figure 11A). The column was then cleaned in place (CIP) using a solution of 0.5 mol/L NaOH. This cleaning procedure was repeated once each day for a total of 15 days. The resolution after this cleaning protocol was equivalent to the resolution of the initial injection of the compounds on the column (Figure 11B).

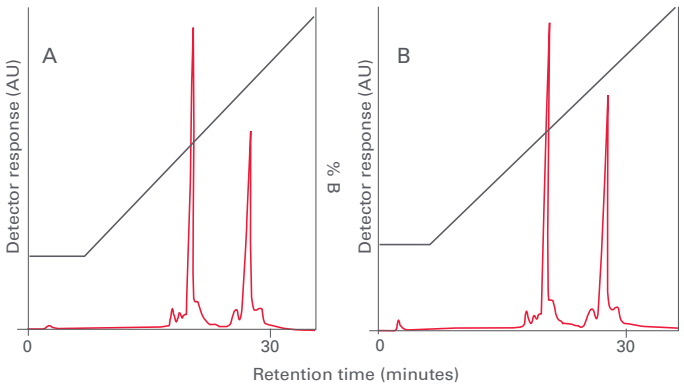
TSKgel 5PW APPLICATIONS

Analysis of *E. coli* RNA

Figure 12 shows the fractionation of high molar mass *E. coli* RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of the TSKgel 5PW resin.

FIGURE 11

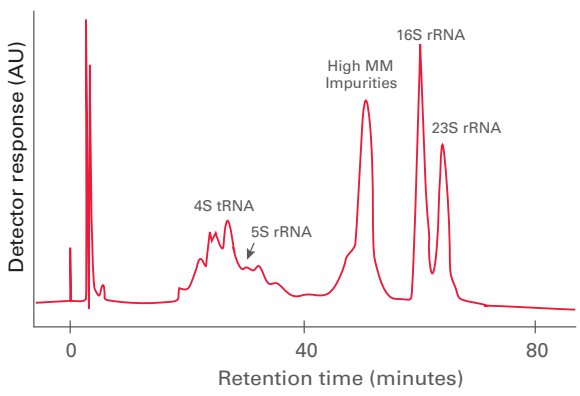
STABILITY OF TSKgel SuperQ-5PW COLUMNS



Column:	TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm L
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.6
	B: 0.5 mmol/L sodium cloride in 50 mmol/L Tris-HCl, pH 8.6
Gradient:	A-B (60 min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	100 µL
Sample load:	each of 1 mg
Samples:	1. ovalbumin
	2. trypsin inhibitor
Note:	A: before CIP
	B: after 15 times (15 days)

FIGURE 12

ANALYSIS OF HIGH MOLAR MASS RNA



Column:	TSKgel DEAE-5PW, 10 µm, 6 mm ID × 15 cm L (custom)
Mobile phase:	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:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	total E. coli RNA

IEC
ABOUT TSKgel DEAE-2SW/-3SW

TSKgel DEAE-2SW and DEAE-3SW columns are packed with porous spherical silica beads which are chemically modified with a weak anion exchange group. These columns are for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins. TSKgel DEAE-2SW columns provide high performance separations of small ionic solutes. The 25 nm pore size TSKgel DEAE-3SW column is used for separatin g peptides, low MW proteins and DNA fragments. The increased solubility of the silica backbone above pH 7 limits the use of the TSKgel SW-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.

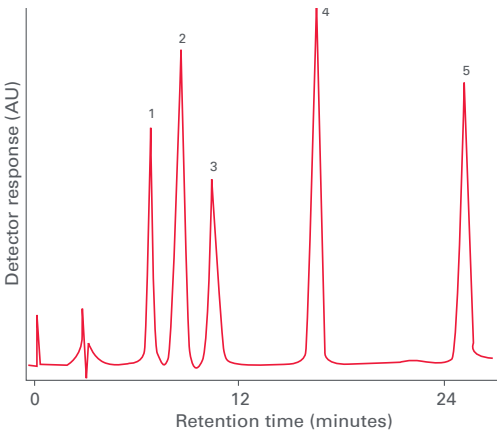
TSKgel DEAE-2SW/3SW APPLICATIONS

Separation of Nucleotides

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 13.

FIGURE 13

SEPARATION OF NUCLEOTIDES



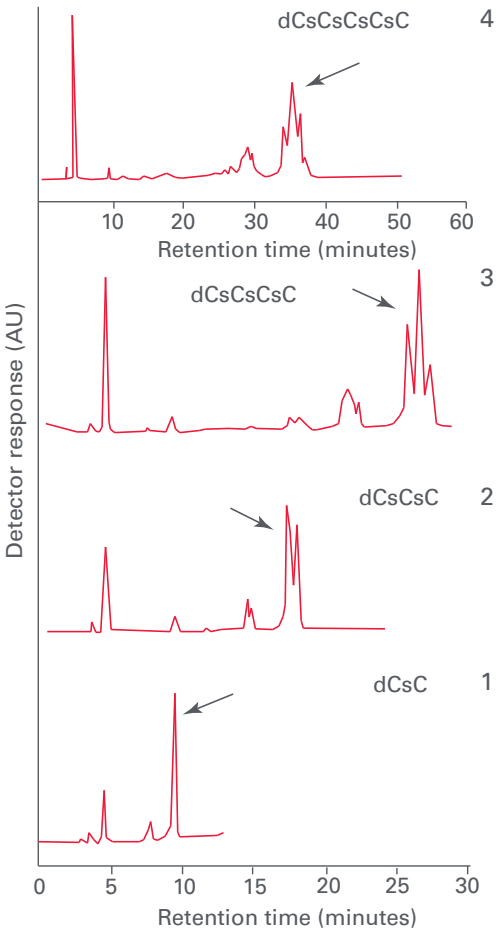
Column:	TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm L
Mobile phase:	A: CH_3CN in 0.1 mol/L phosphate, pH 3.0, 20/80
	B: CH_3CN in 0.5 mol/L phosphate, pH 3.0, 20/80
Gradient:	30 min linear gradient from buffer A to B
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Samples:	1. AMP 2. IMP 3. GMP 4. ADP 5. ATP

Modified Oligonucleotides

Backbone-modified oligonucleotides are increasingly used for antisense therapy. These novel oligos have longer half-lives due to resistance to endogenous nucleases. One common type of backbone-modified oligonucleotides is phosphorothioates where one of the two non-bridged oxygen atoms of the phosphates has been replaced by a sulfur atom. The separation of several phosphorothioates on TSKgel DEAE-2SW is shown in Figure 14.

FIGURE 14

SEPARATION OF PHOSPHOROTHIOATES



Column:	TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm L
Mobile phase:	A: 50 mmol/L ammonium acetate
	B: 1.5 mol/L ammonium acetate
Gradient:	linear, 0-100% B in 60 minutes
Flow rate:	1 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. 2 base phosphorothioate oligonucleotide
	2. 3 base phosphorothioate oligonucleotide
	3. 4 base phosphorothioate oligonucleotide
	4. 5 base phosphorothioate oligonucleotide

IEC
ABOUT TSKgel SPECIALTY AEX COLUMNS

TSKgel Sugar AXG and Sugar AXI columns are specialty columns for the analysis of mono- and disaccharides, as well as sugar alcohols. Both columns are packed with porous spherical PS-DVB polymer beads which are surface modified with a strong anion exchange group.

The TSKgel Sugar AXG column contains 10 µm particles for the gradient separation and analysis of monosaccharides, disaccharides, and sugar alcohols, whereas the TSKgel Sugar AXI column is packed with 8 µm particles for the isocratic separation of carbohydrates where lower and constant back pressures may be generated.

TSKgel SAX columns are packed with 5 µm porous spherical polymer beads which are surface modified with a strong anion exchange group. They are used for the separation of isomerized sugars, alcohols, and low molar mass organic acids.

SPECIALTY COLUMNS APPLICATIONS

Analysis of Saccharides

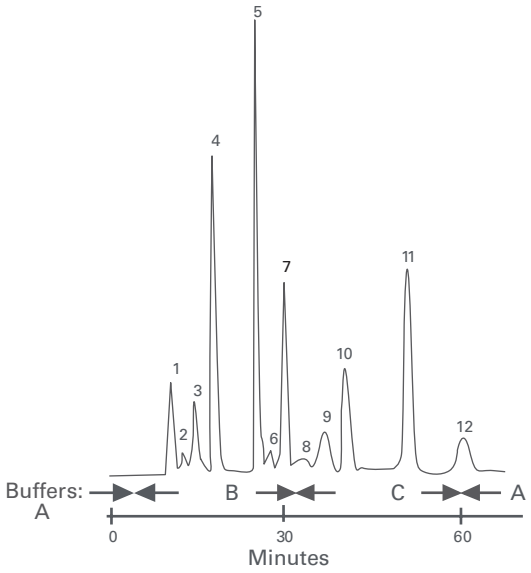
Analyses of monosaccharides, disaccharides, and sugar alcohols can be performed on PS-DVB columns, either by isocratic (TSKgel Sugar AXI) or by gradient (TSKgel Sugar AXG) analysis. Saccharides are retained on Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. Figure 15 shows the separation of twelve mono- and disaccharides on TSKgel SugarAXG.

Sugar Alcohol

Palatinit is a sugar alcohol used as a low-calorie and anti-decay food additive. It can be obtained by reducing palatinose and is composed of two isomers, 6-O-alpha-D-Glucopyranosyl-D-glucitol and 1-O-alpha D-glucopyranosyl-D-mannitol. As shown in Figure 16, a TSKgel Sugar AXG column can separate the isomers.

FIGURE 15

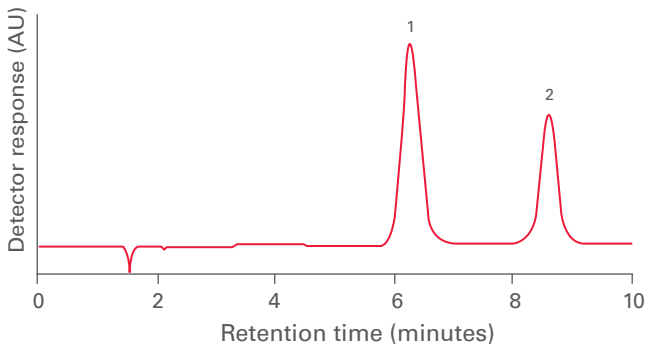
SEPARATION OF SACCHARIDE MIXTURE ON TSKgel Sugar AXG



Column:	TSKgel Sugar AXG, 4.6 mm ID x 15 cm L
Mobile phase:	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/cm²
Temperature:	70 °C (column), 100 °C (post column reactor);
Detection:	fluorescence excitation @331 nm, emission @383 nm
PC reagent:	2.5 % 2-cyanoacetamide solution
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

FIGURE 16

ANALYSIS OF PALATINIT



Column:	TSKgel Sugar AXG, 10 µm, 4.6 mm ID × 15 cm L
Mobile phase:	0.7 mol/L borate buffer, pH 8.6
Flow rate:	0.8 mL/min
Detection:	RI
Temperature:	65 °C
Injection vol.:	10 µL
Samples:	1. alpha-D-glucopyranosyl-1,6-soribitol (GPS) 2. alpha-D-glucopyranosyl-1,6-mannitol (GPM)

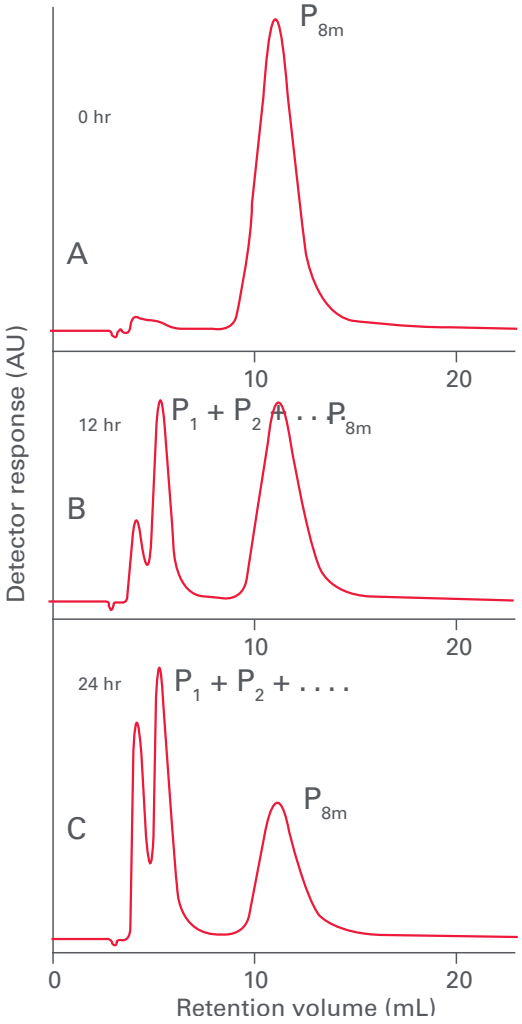
IEC
ABOUT TSKgel SPECIALTY AEX COLUMNS

Polyphosphates

The stability of the TSKgel SAX column allows a wide pH range for separations of polyphosphates. Figure 17 shows the monitoring of cyclooctaphosphate hydrolysis products over the course of 24 hours with a pH 10.2 mobile phase.

FIGURE 17

HYDROLYSIS PRODUCTS OF CYCLOOCTAPHOSPHATE



Column: TSKgel SAX, 5 µm, 4 mm ID × 25 cm L
Mobile phase: 0.4 mol/L KCl, 0.1% EDTA, pH 10.2
Sample: cyclooctaphosphate hydrolysis products
A. 0 hours; B. 12 hours; C. 24 hours

IEC
ORDERING INFORMATION TSKgel AEX COLUMNS

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel AEX columns - silica-based						
0018761	DEAE-2SW	2.0	25.0	5	≥ 5,000	13.0
0007168	DEAE-2SW	4.6	25.0	5	≥ 5,000	15.0
0007163	DEAE-3SW	7.5	7.5	10	≥ 1,300	2.0
TSKgel AEX columns - polymer-based						
0013075	DEAE-NPR, non-porous	4.6	3.5	2.5	≥ 1,300	20.0
0018249	DNA-NPR, non-porous	4.6	7.5	2.5	≥ 6,000	30.0
0021960	Q-STAT, non-porous	3.0	3.5	10	> 200	10.0
0021961	Q-STAT, non-porous	4.6	10.0	7	> 4,000	10.0
0021962	DNA-STAT, non-porous	4.6	10.0	5	> 4,000	15.0
0018757	DEAE-5PW	2.0	7.5	10	≥ 1,300	1.5
0007164	DEAE-5PW	7.5	7.5	10	≥ 1,300	1.5
0007574	DEAE-5PW	21.5	15.0	13	≥ 3,000	2.5
0018257	SuperQ-5PW	7.5	7.5	10	≥ 1,300	2.0
0018387	SuperQ-5PW	21.5	15.0	13	≥ 3,000	2.0
0019685	BioAssist Q	4.6	5.0	10	≥ 500	2.5
0021410	BioAssist Q	10.0	10.0	13	≥ 500	2.5
0008639	Sugar AXI	4.6	15.0	8	≥ 3,700	3.0
0008640	Sugar AXG	4.6	15.0	10	≥ 2,700	2.0
0007157	SAX	6.0	15.0	5	≥ 2,000	15.0
Guardcolumns						
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID guard cartridges	
0017088	DEAE-NPR Guardcolumn	4.6	0.5	5	For P/N 0013075	
0018253	DNA-NPR Guardcolumn	4.6	0.5	2.5	For P/N 0018249	
0007648	DEAE-SW Guardgel Kit			10	For P/Ns 0007168 and 0007163	
0007210	DEAE-5PW Guardgel Kit			20	For P/N 0007164	
0016092	DEAE-5PW Prep Guardgel Kit			20	For P/N 0007574	
0018388	SuperQ-5PW Guardgel Kit			20	For P/N 0018257	
Every Guardgel Kit contains Guardgel, Gelholder and Connector						
TSKgel PW AEX Glass Columns						
0013061	DEAE-5PW Glass	5.0	5.0	10	≥ 700	1.5
0008802	DEAE-5PW Glass	8.0	7.5	10	≥ 1,300	1.0
0014016	DEAE-5PW Glass	20.0	15.0	13	≥ 3,000	1.5
0018386	SuperQ-5PW Glass	8.0	7.5	10	≥ 1,300	2.0
Glass Guardcolumns						
0008806	DEAE-5PW Guardgel Kit, Glass		20		For P/Ns 0013061 and 0008802	
0014466	DEAE-5PW Guardcolumn, Glass	20.0	2.0	13	For P/N 0014016	
Every Guardgel Kit contains Guardgel, Gelholder and Connector						

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ABOUT TSKgel CATION EXCHANGE COLUMNS

- TSKgel CM-STAT columns are ideally suited for antibody charge variant analysis
- TSKgel STAT non-porous 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

TSKgel STAT CATION EXCHANGE COLUMNS

These are non-porous polymer columns with high surface density of carboxymethyl (CM-STAT) and sulfopropyl (SP-STAT) functional groups. Particle sizes and dimensions are optimized either for highest throughput or for highest efficiency. Applications for the TSKgel STAT columns include the separation of peptides, proteins, protein aggregates, charge isomers of monoclonal antibodies and PEGylated proteins.

TSKgel SP-5PW AND CM-5PW

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a strong and a weak cation exchanger, respectively. Proteins and peptides are typical samples that are analyzed on the polymer-based TSKgel cation exchange columns.

TSKgel BioAssist CATION EXCHANGE COLUMNS

These columns are also based on methacrylate particle design technology. TSKgel BioAssist S columns are packed with particles possessing 130 nm pores functionalized with sulfopropyl groups. TSKgel BioAssist columns are available exclusively in PEEK housing.

TSKgel SP-NPR CATION EXCHANGE COLUMNS

Polymethacrylate is the backbone of these non-porous resin columns, which 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. Due to the absence of large pores, protein recovery is generally very high on TSKgel NPR columns.

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

Silica-based TSKgel cation exchange columns with sulfopropyl (SP) and carboxymethyl (CM) functional groups are available for analyzing smaller molar mass samples such as drug candidates and small peptides or proteins. Binding capacity for small to medium size proteins on these columns is approximately double that of the TSKgel 5PW packings due to the smaller pore size and larger surface area.

SPECIALTY TSKgel POLYSTYRENE-BASED CATION EXCHANGE COLUMNS

Strong cation exchange TSKgel SCX columns are available for the analysis of organic acids, saccharides and alcohols.

IEC
ABOUT TSKgel CATION EXCHANGE COLUMNS

Tables V and VI summarize the features and benefits of TSKgel cation exchange columns according to matrix and list the properties of available columns.

TABLE V

FEATURES AND BENEFITS OF TSKgel CATION EXCHANGE COLUMNS

TSKgel Column Type	Type/Matrix	Benefit
CM-STAT, SP-STAT	strong(SP-STAT), weak (CM-STAT)/polymer	Non-porous with high surface density of carboxymethyl (CM) and sulfopropyl (SP) groups
CM-5PW, SP-5PW	strong (SP-5PW), weak (CM-5PW)/polymethacrylate	Polymethacrylate resin derivatized with carboxymethyl (CM) and sulfopropyl (SP) groups
BioAssist S	strong/polymethacrylate	Contain very large pores (130 nm), resulting in high binding capacity and improved recovery of activity; available exclusively in PEEK housing
SP-NPR	strong/polymethacrylate	Non-porous with 2.5 µm particles; fast analysis; high protein recovery
CM-2SW, CM-3SW, SP-2SW	strong (SP-2SW), weak (CM-2SW, CM-3SW)/silica	Silica-based with carboxymethyl (CM) and sulfo-propyl (SP) functional groups
SCX	strong (SCX) Polystyrene Divinyl Benzene (PS-DVB)	Specialty columns for the analysis of organic acids, saccharides and alcohols

TABLE VI

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 = polymethacrylate; PS-DVB = polystyrene-divinylbenzene*
** *Polyethylene glycol*
*** *PEEK = polyethyletherketone, S = stainless steel, G = glass*
(1) γ-globulin; (2) hemoglobin; (3) lysozyme

IEC
ABOUT TSKgel SP-/CM-STAT

TSKgel CM-STAT and SP-STAT columns are packed with 7 or 10 µm hydrophilic non-porous resin particles of which the surface consists of an open access network of multi-layered weak (CM-STAT) or strong (SP-STAT) cation exchange groups (see Figure 18). The innovative bonding chemistry, combined with a relatively large particle size, results in a respectable loading capacity, low operating pressure, and rapid analysis.

TSKgel STAT cation exchange columns are available in various column formats and particle sizes to perfectly match specific application needs. For fast and ultra-fast 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. Applications for the TSKgel CM-STAT and SP-STAT columns include the separation of proteins, protein aggregates, charge variants of monoclonal antibodies, PEGylated proteins, and peptide digests.

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

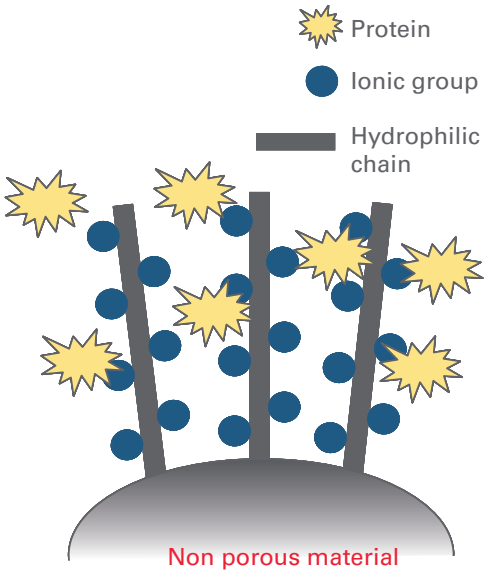
TABLE VII

BASIC PROPERTIES OF TSKgel STAT CATION EXCHANGE COLUMNS

Property	TSKgel SP-STAT		TSKgel CM-STAT	
Base material	Cross-linked hydrophilic polymer (monodisperse particles)			
Pore size	Non-porous			
Functional group	Sulfopropyl		Carboxymethyl	
Particle size	7 µm	10 µm	7 µm	10 µm
Column size (mm ID x cm L)	4.6 x 10	3 x 3.5	4.6 x 10	3 x 3.5
Application	High resolution protein separation	High through-put protein separation		

FIGURE 18

SCHEMATIC DIAGRAM OF TSKgel STAT SERIES



IEC
TSKgel SP-/CM-STAT APPLICATIONS

ANALYSIS OF mAb CHARGE VARIANTS

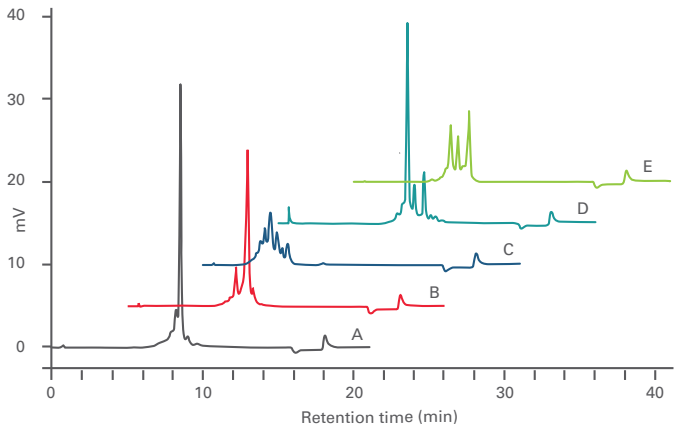
TSKgel CM-STAT columns are ideally suited to analyze the profile of charge isomers of proteins. Figure 19 shows the analysis profiles for five antibodies and their charge isomers separated on a TSKgel CM-STAT column.

MONITORING OF PEGYLATION

TSKgel STAT columns provide fast, high resolution separations at moderate pressures. Figure 20 shows the monitoring of a PEGylation reaction of beta-lactoglobulin on a short SP-STAT column. Analysis is performed in less than 3 minutes.

FIGURE 19

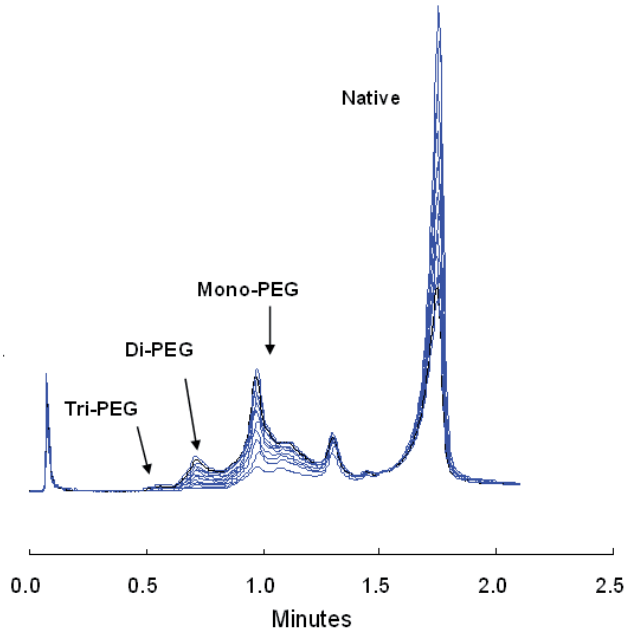
SEPARATION OF mAb CHARGE VARIANTS ON TSKgel CM-STAT



Column:	Prototype SP-STAT, 10 µm, 4.6 mm ID x 3.5 cm L
Mobile Phase:	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 20

MONOTORING OF PEGYLATION OF β-LACTOGLOBULIN



Column:	TSKgel CM-STAT, 7 µm, 4.6 mm ID x 10 cm L
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
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol.:	20 µL

IEC
ABOUT TSKgel SP-NPR

The TSKgel SP-NPR column is packed with spherical, non-porous (NPR) hydrophilic polymer beads of which the surface has been modified with a strong cation exchange group. Non-porous resin columns provide fast separations due to their small (2.5 µm) particle size.

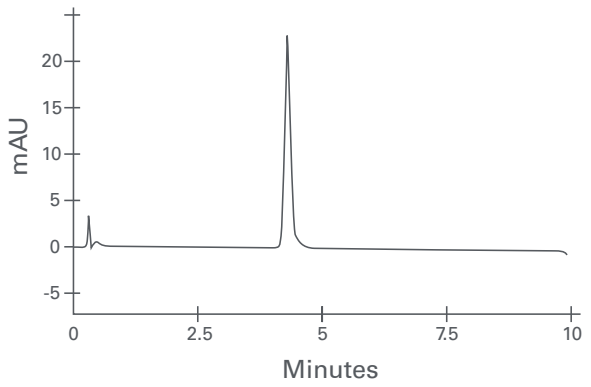
The TSKgel SP-NPR column is used for the separation and analysis of proteins and peptides. This column is particularly useful for very large biopolymers.

TSKgel SP-NPR APPLICATIONS

Purity Analysis of Adeno-Associated Viruses
TSKgel SP-NPR columns provide fast separations due to their small spherical particles. A purity check of adeno-associated virus, commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in Figure 21. This ten minute HPLC method replaces an existing assay that took two days.

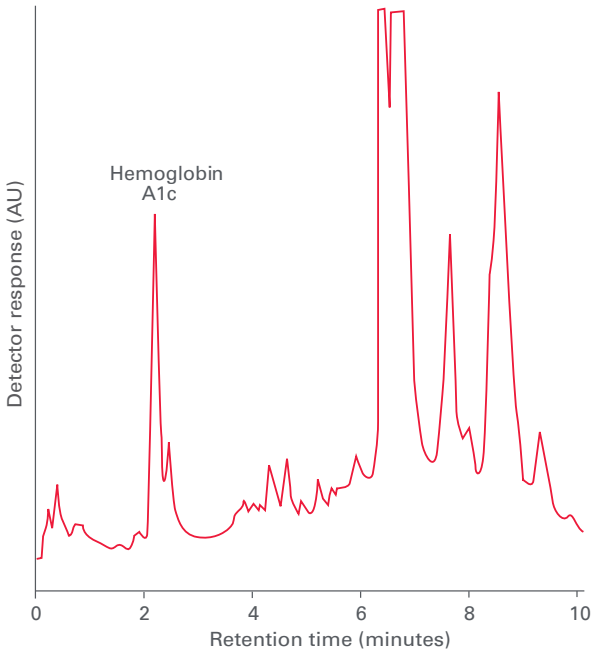
Analysis of Hemoglobin A1c level
The analysis of hemoglobin A1c levels in blood is used to monitor glucose levels in diabetic patients. Figure 22 shows that the HbA1c fraction can be separated from other human Hb variants on a TSKgel SP-NPR column by running a linear pH gradient in ten minutes.

FIGURE 21
ANALYSIS OF PURIFIED AAV WITH TSKgel SP-NPR



Column:	TSKgel SP-NPR, 4.6 mm ID x 3.5 cm L
Mobile phase:	A. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L $MgCl_2$, pH 7.5; B. 50mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L $MgCl_2$, 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
Sample:	purified adeno-associated virus

FIGURE 22
pH GRADIENT ANALYSIS OF HEMOGLOBIN A1c



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

IEC
ABOUT TSKgel BIOASSIST S

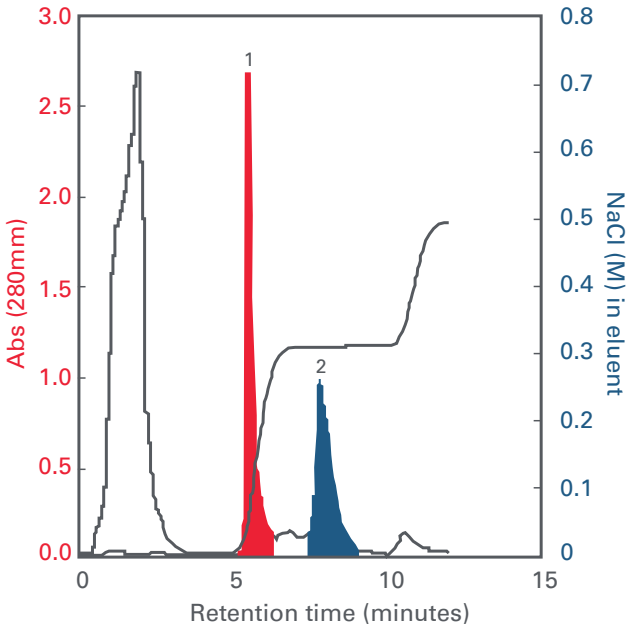
Specially designed for the separation of large biomolecules such as antibodies, the large pores of the TSKgel BioAssist S cation exchange column offer superior capacity and resolution at a low column pressure drop. The polymerization technique used to create this stationary phase results in an equivalent density of ionic exchange groups to be incorporated into the polymethacrylate particle without reducing pore size.

The TSKgel BioAssist S columns' large pores are very accessible even for high molar mass proteins. This leads to higher chromatographic efficiency and binding capacity for purification.

TSKgel BioAssist S cation exchange columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale up. Both columns are made of PEEK to reduce protein adsorption. TSKgel BioAssist S columns are suitable for use in systems that are designed for HPLC, laboratory, or semi-preparative applications.

FIGURE 23

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

TSKgel BioAssist S APPLICATIONS

Immunoglobulin M (IgM)

gM 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 thiophilic absorbents but these methods often result in low binding capacity.

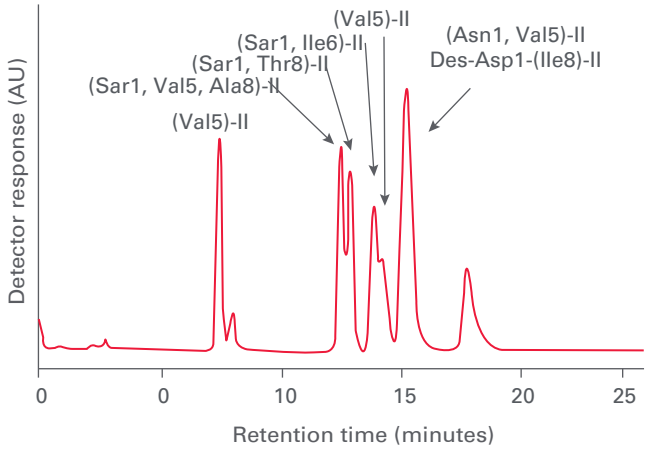
An alternative purification method of IgM by ion exchange chromatography using a TSKgel BioAssist S column was developed. As shown in Figure 23, baseline separation of IgM from other contaminants is achieved using a 0.3 mol/L NaCl step gradient after elution of albumin.

Peptides

Figure 24 shows chromatograms of peptides on a TSKgel BioAssist S column. It is generally known that an accurate quantification is difficult to obtain when peptides are analyzed on a column with a styrene-type base material, due to secondary interaction with the hydrophobic packing material. However, a TSKgel BioAssist S column is capable of analyzing such peptides as angiotensins without the need to add an organic solvent to the mobile phase since the acrylate packing material is hydrophilic.

FIGURE 24

ANALYSIS OF PEPTIDES



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

IEC
ABOUT TSKgel SP-/CM-5PW

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

TSKgel CM-5PW columns are used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are offered in dimensions of 7.5 mm ID × 7.5 cm in stainless steel housing. TSKgel SP-5PW columns are also used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are available in internal diameters varying from 2 mm to 21.5 mm and in column housings of either glass or stainless steel.

DIFFERENCES IN SELECTIVITY

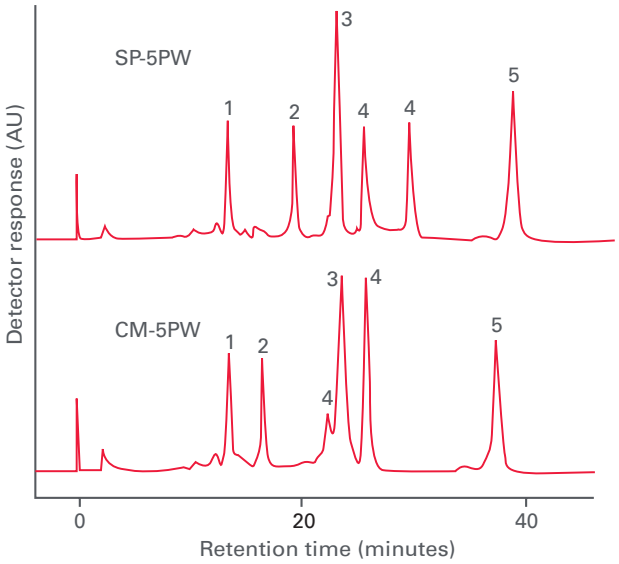
Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchange columns are demonstrated in Figure 25, which is a separation of globular proteins.

TSKgel SP-5PW APPLICATIONS

Purification of Lipoxidase
The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in Figure 26. Scale up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) columns.

FIGURE 25

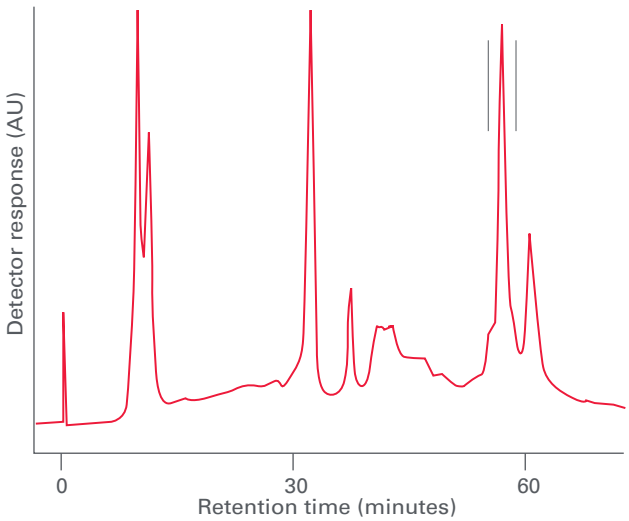
SELECTIVITY OF STRONG AND WEAK TSKgel CATION EXCHANGE COLUMNS



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

FIGURE 26

SEMI-PREPARATIVE PURIFICATION OF LIPOXIDASE



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

IEC
ABOUT TSKgel SP-/CM-2SW AND SP-3SW

The TSKgel SP-2SW, TSKgel CM-2SW, and TSKgel CM-3SW columns are silica-based columns derivatized with sulfopropyl (SP) and carboxymethyl (CM) ligands to provide a strong cation and weak cation exchange column, respectively.

Silica-based cation exchange columns are typically used for the separation and analysis of small proteins, peptides, and other biologically active molecules. TSKgel CM-2SW has a smaller pore size than TSKgel CM-3SW.

TSKgel SP-2SW APPLICATIONS

Herbicides

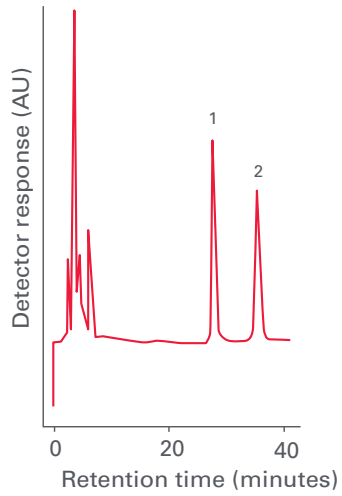
Figure 27 shows the rapid analysis of the herbicides paraquat and diquat in urine on the TSKgel SP-2SW column.

Nucleosides

Figure 28 shows the separation of nucleosides on the TSKgel SP-2SW column.

FIGURE 27

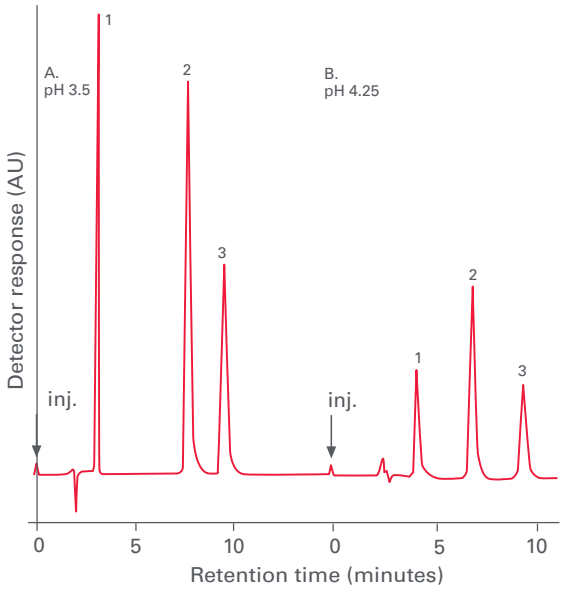
RAPID ANALYSIS OF PARAQUAT AND DIQUAT



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

FIGURE 28

SEPARATION OF NUCLEOSIDES



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

IEC
ABOUT TSKgel SPECIALTY CEX COLUMNS

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

The TSKgel SCX column is also available in the H^+ form for the separation of isomerized sugars, alcohols, and lower organic acids.

COLUMN STABILITY

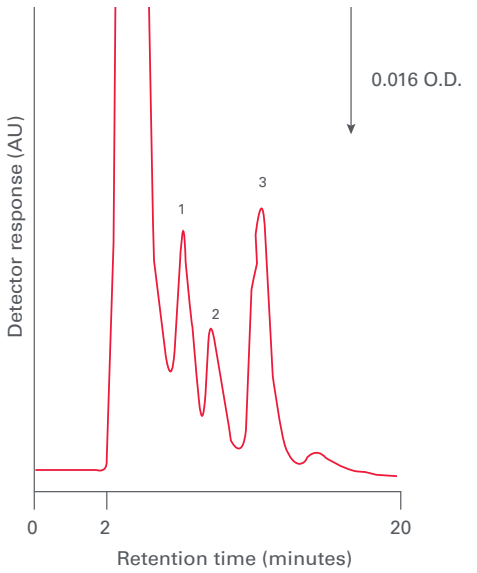
An example of the stability of the TSKgel SCX column is demonstrated in Figure 29 where 1 mol/L NaOH is used as the mobile phase for the separation of organic acids.

TSKgel SCX APPLICATIONS

Saccharide, Organic Acid, and Alcohol Mixture
on exclusion chromatography can be used as an effective method for separating alcohols. An example of saccharide, organic acid, and alcohol separation is shown in Figure 30 on two TSKgel SCX (H^+) columns in series.

FIGURE 29

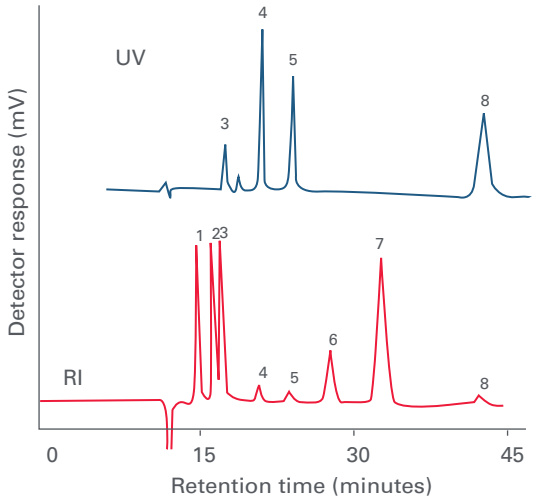
SEPARATION OF ACIDS



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

FIGURE 30

SEPARATION OF SACCHARIDE, ORGANIC ACID, AND ALCOHOL MIXTURE



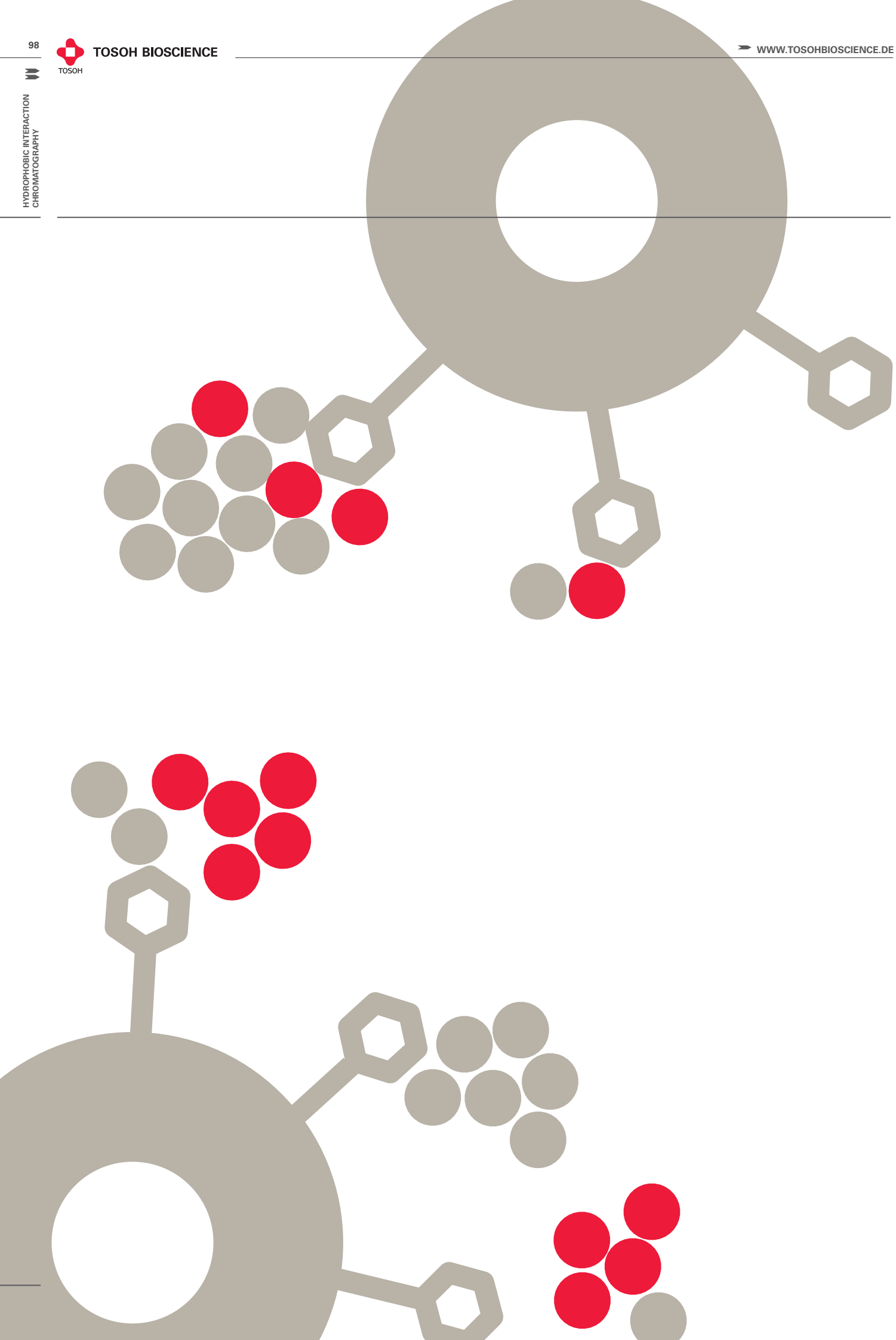
Column:	TSKgel SCX (H+), 5 µm, 7.8 mm ID × 30 cm L × 2
Mobile phase:	0.05 mol/L $HClO_4$
Flow rate:	0.8 mL/min
Detection:	UV @ 210 nm, RI
Samples:	1. maltose
	2. glucose
	3. fructose
	4. lactic acid
	5. acetic acid
	6. methanol
	7. ethanol
	8. butyric acid

IEC
ORDERING INFORMATION TSKgel CATION EXCHANGE

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel CEX Columns - silica-based						
0007167	CM-2SW	4.6	25.0	5	≥ 5,000	15.0
0007162	CM-3SW	7.5	7.5	10	≥ 1,300	2.0
0007165	SP-2SW	4.6	25.0	5	≥ 5,000	15.0
TSKgel CEX Columns - polymer-based						
0013068	CM-5PW	7.5	7.5	10	≥ 1,300	1.5
0013076	SP-NPR, non-porous	4.6	3.5	2.5	≥ 1,300	20.0
0021963	SP-STAT, non-porous	3.0	3.5	10	≥ 200	10.0
0021964	SP-STAT, non-porous	4.6	10.0	7	≥ 200	10.0
0021965	CM-STAT, non-porous	3.0	3.5	10	≥ 200	10.0
0021966	CM-STAT, non-porous	4.6	10.0	7	≥ 2,000	10.0
0018758	SP-5PW	2.0	7.5	10	≥ 1,300	1.0
0007161	SP-5PW	7.5	7.5	10	≥ 1,300	1.5
0007575	SP-5PW	21.5	15.0	13	≥ 3,000	2.5
0019686	BioAssist S PEEK	4.6	5.0	7	≥ 1,500	2.5
0021411	BioAssist S PEEK	10.0	10.0	13	≥ 3,000	2.5
0007156	SCX (Na+)	6.0	15.0	5	≥ 2,000	15.0
0007158	SCX (H+)	7.8	30.0	5	≥ 12,000	5.0
Guardcolumns						
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID guard cartridges	
0007650	CM-SW Guardgel Kit			20	For P/Ns 0007167 and 0007162	
0013069	CM-5PW Guardgel Kit			10	For P/N 0013068	
0016093	SP-5PW Prep Guardgel Kit			20	For P/N 0007575	
0007211	SP-5PW Guardgel Kit			20	For P/N 0007161	
Every Guardgel Kit contains Guardgel, Gelholder and Connector						
TSKgel PW-CEX Glass Columns						
0013062	SP-5PW Glass	5.0	5.0	10	≥ 700	1.5
0008803	SP-5PW Glass	8.0	7.5	10	≥ 1,300	1.0
0014017	SP-5PW Glass	20.0	15.0	13	≥ 3,000	1.5
Guardcolumns						
0008807	SP-5PW Guardgel Kit, Glass			20	For P/Ns 0013062 and 0008803	

Every Guardgel Kit contains Guardgel, Gelholder and Connector



HIC HYDROPHOBIC INTERACTION CHROMATOGRAPHY

HIC PRODUCTS

POLYMER BASED HIC COLUMNS

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

Tosoh Bioscience is the sole sponsor of the HIC-DSP conference.

The intimate character of the conference offers an unparalleled opportunity to network and exchange scientific ideas. Better than other conferences attended.

More information: www.hic-dsp.org

HIC HIGHLIGHTS

HIGHLIGHTS TSKgel Butyl-NPR

- TSKgel Butyl-NPR columns support easy method transfer from HPLC to UHPLC
- The proven Butyl-NPR selectivity delivers efficient DAR analysis of ADCs
- High speed and high resolution analysis with HPLC and UHPLC systems

FEATURES

- Choice of three hydrophobic ligands
- Rigid polymeric base resins
- Some columns offered in PEEK hardware
- Same chemistry as TOYOPEARL resins

BENEFITS

- Cover a wide spectrum of sample polarities
- Wide buffer pH (2-12) range
- Eliminates undesirable interactions
- Seamless scalability from analytical to preparative scale

HIC
HOW DOES IT WORK?

Hydrophobic Interaction Chromatography (HIC) is used primarily for the separation of non-polar and hydrophobic compounds under non-denaturing conditions. HIC is based on non-polar interactions that are induced by high salt mobile phases. Stationary phases are similar to reversed phase chromatography (RPC) but the density of functional groups is lower.

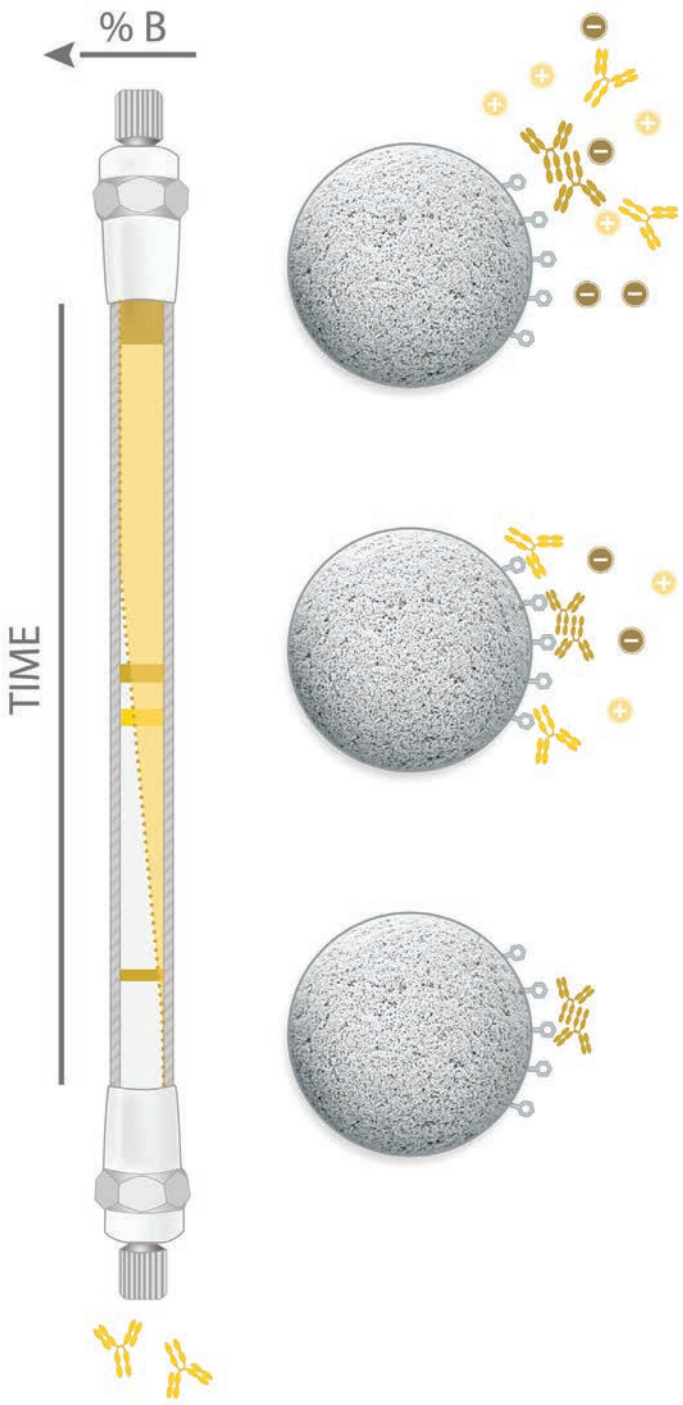
Proteins and other molecules with hydrophobic surfaces are attracted to the hydrophobic ligands of both reversed phase and HIC stationary phases. RPC phases have higher surface coverage and/or more hydrophobic ligand compared to HIC phases. Because of this, in a RPC separation the target binding readily occurs in an aqueous solution, and desorption is promoted by the addition of an increasing amount of organic solvent.

In HIC, proteins are bound to the particle by employing an aqueous high salt mobile phase. The salt conditions contribute to a lyotropic effect which allows the proteins to bind to the lower surface coverage of a hydrophobic ligand. Proteins are separated by the simple technique of decreasing the salt concentration. Since HIC separates under milder eluting conditions, biological activity is typically retained.

HIC is used in the biopharmaceutical industry for the analysis of antibody drug conjugates (ADCs) or as an orthogonal method to SEC to determine the aggregate content of monoclonal antibodies.

FIGURE 1

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ILLUSTRATION



HIC STATIONARY PHASES

TSKgel HIC columns are polymethacrylate-based with a choice of three ligands (butyl, ether, and phenyl) with varied hydrophobicities from low to high. This enables the user to perfectly match HIC selectivity to specific application needs.

PACKING MATERIALS AND CHEMISTRIES

The HIC packing materials are based on the polymeric TSKgel G5000PW resin which is then derivatized with oligoethylene-glycol (Ether-5PW) or phenyl (Phenyl-5PW) groups. The base material used to prepare TSKgel Butyl-NPR consists of spherical 2.5 µm non-porous particles. Non-porous resins (NPR) are typically used for high speed analytical applications. The TSKgel HIC columns are compatible with water-soluble organic solvents at concentrations below 50% (20% for TSKgel Butyl-NPR).

COLUMN SELECTION

TSKgel Butyl-NPR is the least hydrophobic HIC column in the TSKgel HIC series and requires a higher salt concentration for binding. It is an excellent choice for monoclonal antibody analysis and high speed applications. TSKgel Butyl-NPR is getting increasingly popular for the analysis of antibody-drug conjugates (ADCs).

TSKgel Ether-5PW provides an intermediate hydrophobicity and is an excellent choice for hydrophobic proteins such as membrane proteins or monoclonal antibodies. Because of the porous base matrix it can be used for larger amounts of sample to be analyzed and capacity of Butyl-NPR is too small.

TSKgel Phenyl-5PW is the most hydrophobic phase in the TSKgel HIC series and thus requires only modest salt concentration to retain proteins. It is applicable for the widest range of sample hydrophobicities.

Table I lists well-known applications for HIC columns. Figure 3 compares the separation of standard proteins on the Ether, Phenyl, and Butyl columns under similar operating conditions.

FIGURE 2

STRUCTURE OF TSKgel HIC PHASES

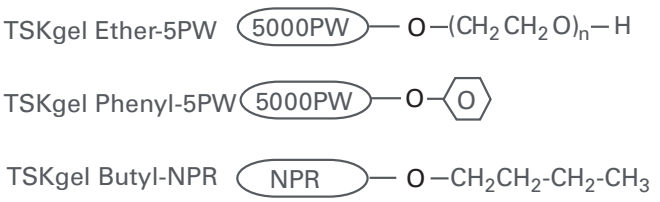
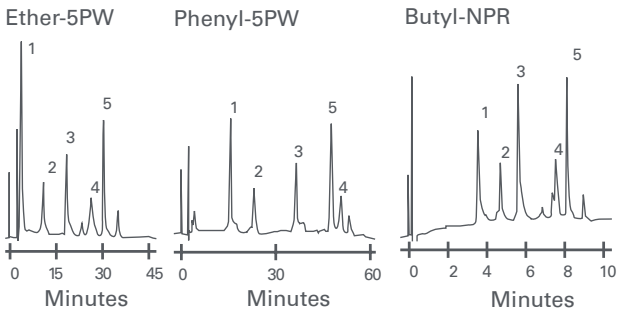


FIGURE 3

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

Injection vol.: 5PW-type columns: 100 µL (50-100 µg),
NPR-type column: 20 µL (1.5-40 µg)

Sample:
1. myoglobin,
2. ribonuclease A,
3. lysozyme,
4. α-chymotrypsin,
5. α-chymotrypsinogen

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

HIC
ABOUT TSKgel BUTYL-NPR

- Optimized for efficient analysis of antibody-drug-conjugates
- Excellent recovery allows quantitation down to nanogram levels
- Stable in wide pH range

TSKgel ButyL-NPR PROPERTIES

The 2.5µm non-porous methacrylate packing material of the TSKgel Butyl-NPR columns is bonded with butyl groups. In terms of hydrophobicity, the TSKgel Butyl-NPR columns are the least hydrophobic of the HIC column offerings and require a higher salt concentration for binding. They are the best choice for high speed separations with excellent recovery, even for more hydrophobic samples.

As in other modes of liquid chromatography, smaller particles provide higher efficiency. By packing the 2.5µm non-porous resin particles into shorter columns, typical analysis times are reduced to less than ten 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. Because the surface area of non-porous particles is much smaller, sample amount and volume need to be adjusted to maintain optimum column efficiency.

TSKgel Butyl-NPR is available in two dimensions: 3.5 cm length for high throughput and 10 cm length for high resolution.

TSKgel Butyl-NPR APPLICATIONS

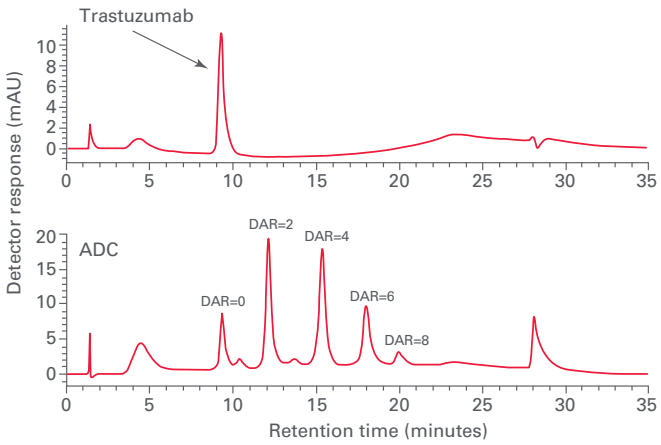
Analysis of DAR of Antibody-Drug Conjugates
Antibody-drug conjugates (ADCs) are becoming an increasingly important class of therapeutic agents for various diseases. One of the most important quality attributes of an ADC is the drug-to-antibody ratio (DAR), the average number of drugs that are conjugated. This determines the amount of "payload" that can be delivered to the target cell e.g. a tumor cell.

Figure 4 shows the HIC analysis of a drug conjugated Trastuzumab. Unconjugated monoclonal antibody (Trastuzumab) and drug conjugated Trastuzumab (Trastuzumabvc-MMAE) samples were injected onto a 10 cm TSKgel Butyl-NPR column. Gradient elution was performed with sodium phosphate buffer/isopropanol (80/20).

The unconjugated Trastuzumab sample elutes as a major single peak at approximately 9.5 minutes (upper panel). This single peak indicated that the unconjugated Trastuzumab consisted of mostly homogeneous molecules. The profile of the drug conjugated Trastuzumab exhibites well resolved peaks with different retention times than that of the unconjugated drug and with baseline separation (lower panel). These well resolved peaks have different drug-to-antibody ratios (DAR). These peaks range in DAR from 0 to 8, estimated based on the retention time of the peaks. Different drug loads cause an increase in hydrophobicity which result in differing elution times; the lower drug-loaded peaks elute first and the higher drug-loaded peaks elute later. The ADC peak with a retention time of 9.5 minutes indicates the presence of a certain amount of unconjugated Trastuzumab (DAR=0).

FIGURE 4

ANALYSIS OF UNCONJUGATED AND DRUG CONJUGATED TRASTUZUMAB



Column:	TSKgel Butyl-NPR, 2.5µm, 4.6 mm ID × 10 cm L
Mobile phase:	A: 25 mmol/L phosphate buffer, pH 7.0,
	+ 1.5 mol/L ammonium sulfate
	B: 25 mmol/L phosphate buffer, pH 7.0,
	+ 2-propanol - (80:20)
Gradient:	0 - 100 % B (20 minutes)
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 µL
Samples:	Trastuzumab, 0.24 g/L
	ADC(Trastuzumab-vcMMAE), 2.2 g/L

HIC
ABOUT TSKgel ETHYL-/PHENYL-5PW

- Alternative hydrophobicities
- BioAssist-Phenyl PEEK column available for sensitive proteins
- Stable in wide pH range

TSKgel Ethyl-5PW AND Phenyl-5PW PROPERTIES

TSKgel Phenyl-5PW columns were the first commercially available, polymer-based columns for high performance HIC. These columns have been instrumental in the increase in popularity of this technique for analytical, preparative, and process scale separations of biopolymers. The high porosity of TSKgel Phenyl-5PW packings allows very large proteins to enter the internal pore structure, thereby maintaining high capacity for such compounds. TSKgel Phenyl-5PW - the most hydrophobic among the three TSKgel HIC columns - are an excellent choice to screen for the selectivity, retention, and recovery of most biomolecules. TSKgel Ether-5PW columns are less hydrophobic than Phenyl-5PW.

TSKgel Ether-5PW and Phenyl-5PW are stable in either acid or caustic cleaning regimens.

TSKgel Ethyl-5PW APPLICATIONS

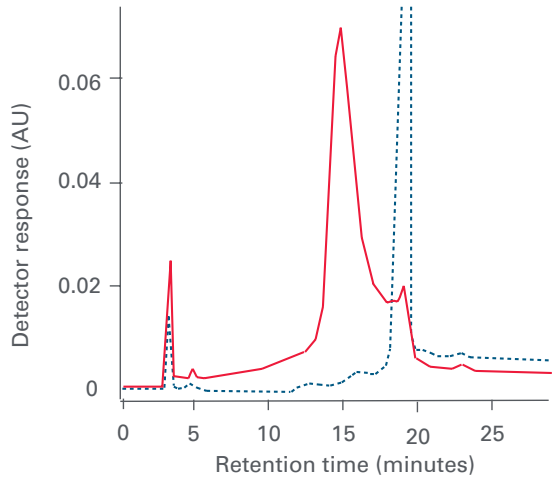
Purity control of an anti-tumor antibiotic
TSKgel Ether-5PW was used to determine the relative purity of the antibiotic components C-1027 and C-1027-AG as shown in Figure 7. Antibiotic C-1027 is composed of a protein consisting of many hydrophobic and hydroxy-amino acids with a non-protein chromophore. Antibiotic C-1027-AG is composed of the hydrophobic and hydroxy-amino acids without the chromophore.

TSKgel Phenyl-5PW APPLICATIONS

Separation of ribosomal RNA
Figure 8 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column. The approximate molar masses of these RNAs are 5.6×10^5 and 1.1×10^6 Da, respectively.

FIGURE 7

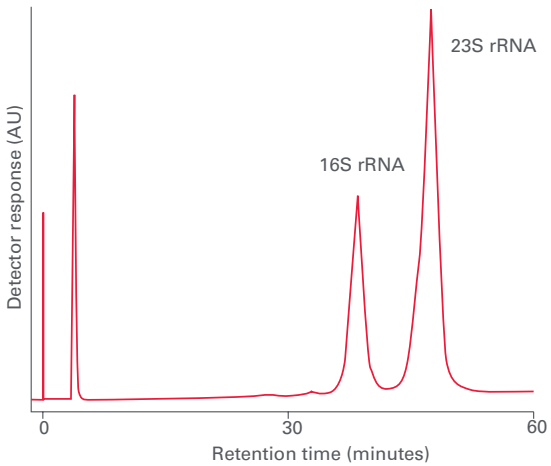
PURIFICATION OF ANTI-TUMOR ANTIBIOTIC



Column: TSKgel Ether-5PW, 10 µm, 7.5 mm ID × 7.5 cm L
Mobile phase: 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
Injection vol.: 20 µL
Sample: — C-1027
···· C-1027-AG
concentration: 1 g/L

FIGURE 8

ANALYSIS OF RIBOSOMAL RNA

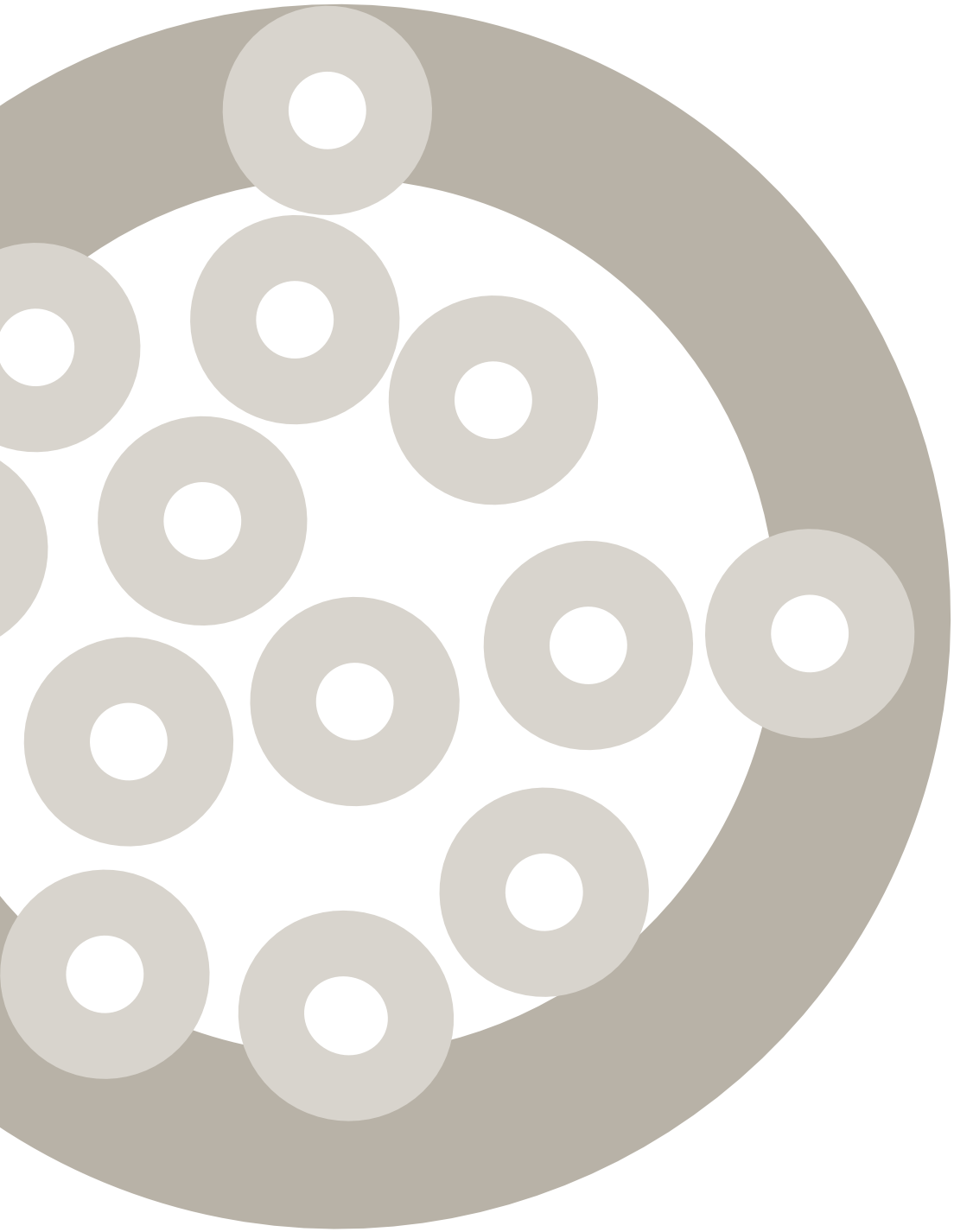


Column: TSKgel Phenyl-5PW, 10 µm, 7.5 mm ID × 7.5 cm LMobile phase: 60 min linear gradient from 2 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 0.5 mL/min
Detection: UV @ 280 nm
Sample: 16S and 23S rRNA from *E. coli*, 0.05 mg in 0.1 mL

HIC
ORDERING INFORMATION TSKgel HIC COLUMNS

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel PW-HIC Columns						
0014947	Butyl-NPR, non-porous	4.6	3.5	2.5		20.0
0042168	Butyl-NPR, non-porous	4.6	10.0	2.5	> 4,000	20.0
0018760	Ether-5PW	2.0	7.5	10.0	≥ 1,000	0.6
0008641	Ether-5PW	7.5	7.5	10.0	≥ 1,000	2.0
0014013	Ether-5PW Glass	5.0	5.0	10.0	≥ 600	2.0
0014014	Ether-5PW Glass	8.0	7.5	10.0	≥ 1,000	2.0
0018759	Phenyl-5PW	2.0	7.5	10.0	≥ 1,000	0.8
0007573	Phenyl-5PW	7.5	7.5	10.0	≥ 1,000	2.0
0007656	Phenyl-5PW	21.5	15.0	13.0	≥ 3,000	2.0
0013063	Phenyl-5PW Glass	5.0	5.0	10.0	≥ 600	2.0
0008804	Phenyl-5PW Glass	8.0	7.5	10.0	≥ 1,000	2.0
0020023	BioAssist Phenyl PEEK	7.8	5	10.0	≥ 1,000	2.0
Guardcolumns						
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID guard cartridges	
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	
Every Guardgel Kit contains Guardgel, Gelholder and Connector						



HILIC HYDROPHILIC INTERACTION CHROMATOGRAPHY

HILIC PRODUCTS

SILICA BASED HILIC COLUMNS

TSKgel Amide-80
TSKgel NH_2-100

What is the difference of HILIC and normal phase chromatography?

Both modes use the same stationary phase. The major differences are the composite of the mobile phase and the mechanism of separation. Normal phase uses 100 % organic mobile phases while HILIC uses organic mobile phases that are water miscible.

HILIC HIGHLIGHTS

HIGHLIGHTS TSKgel Amide-80

- TSKgel Amide-80 2 µm UHPLC columns support easy method transfer from HPLC to UHPLC
- The proven Amide-80 selectivity delivers efficient glycan pattern analysis
- High speed and high resolution analysis with HPLC and UHPLC systems

HIGHLIGHTS TSKgel NH_2-100

- Alternative HILIC selectivity option
- Better durability than traditional amino phases
- A direct connect (DC) version can be connected directly to reversed phase columns

FEATURES

- Choice of two kinds of functional groups
- Stable bonding chemistries
- Proven Amide-80 selectivity in many particle sizes
- Stable in 100 % organic eluents

BENEFITS

- Cover a wide spectrum of sample polarities
- Low bleeding is ideal for mass spec detection
- Enables seamless scalability
- Suitable for both, HILIC and normal phase use

HILIC
HOW DOES IT WORK?

Hydrophilic Interaction Liquid Chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC stationary phases are polar, similar to normal phase chromatography (NPC), but mobile phases are similar to reversed phase chromatography (RPC). Typical mobile phases are aqueous buffers with organic modifiers - primarily acetonitrile - applied in isocratic or gradient mode. Typical HILIC stationary phases are silica or polymer particles carrying polar functional groups, e.g. hydroxyl, carbamoyl, amino or zwitterionic groups.

It is commonly believed that in HILIC the aqueous content of the mobile phase creates a water rich layer on the surface of the stationary phase. This allows for partitioning of solutes between the more organic mobile phase and the aqueous layer. The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determine the elution order. Since the retention is also related to the type of functional groups of the stationary phase, it varies between different HILIC phases.

Compared to RPC the elution order in HILIC mode is inversed for most compounds. The HILIC mode can only be executed when starting at high acetonitrile concentrations and offers unique advantages for mass spectrometric 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. While using similar eluent systems HILIC and reversed phase can also be easily combined for two-dimensional liquid chromatography (2D-LC).

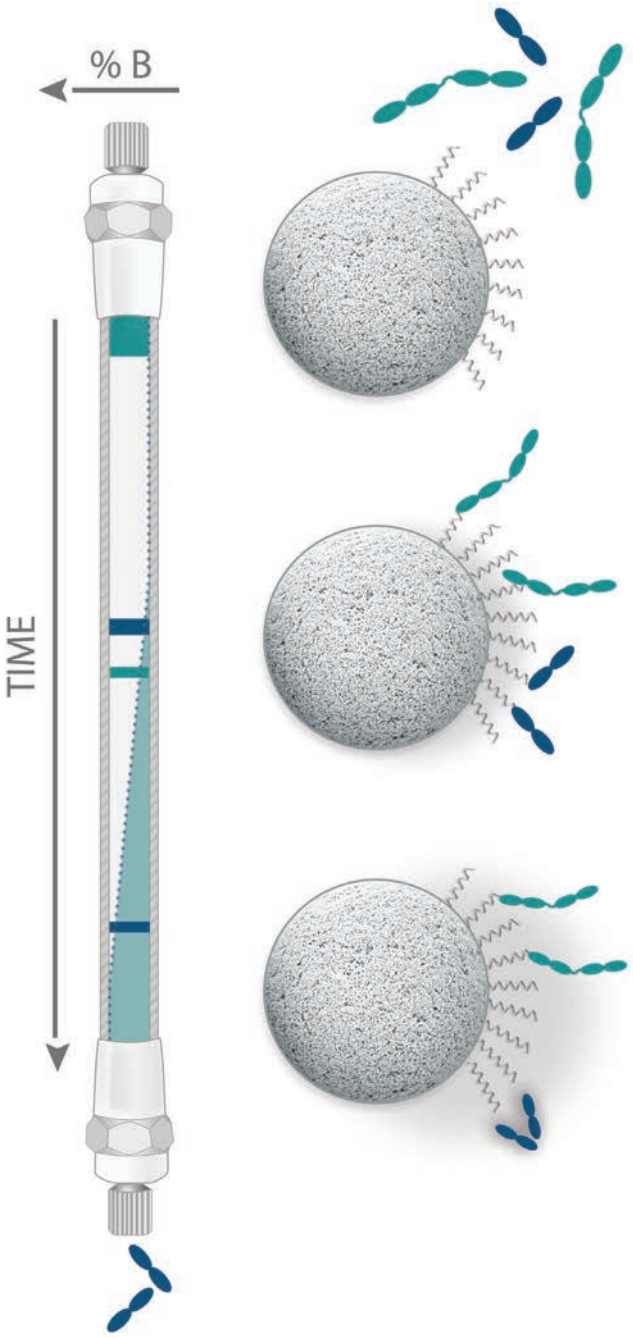
In method development HILIC is an option as soon as polar compounds have to be analyzed and retention on reversed phase columns is too low. Since common RPC solvents can be used, TSKgel HILIC columns can be implemented in method development systems using automated column selection. A choice of reversed phase columns differing in hydrophobicity or carrying polar embedded groups and one of the TSKgel HILIC column types will deliver an indication for the right direction of method development.

TYPICAL APPLICATIONS FOR HILIC ARE:

- Analysis of polyols, carbohydrates, or vitamins
- Characterization of protein glycosylation by fluorescence or mass spectrometric detection
- Separation of polar peptides, e.g. after enzymatic digestion of proteins (peptide mapping)
- Analysis of polar drugs and separation of drug metabolites
- LC/MS analysis of polar compounds

FIGURE 1

HYDROPHILIC INTERACTION CHROMATOGRAPHY ILLUSTRATION



HILIC STATIONARY PHASES

TSKgel HILIC columns are available in various dimensions and particle sizes. They are based on silica particles 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.

PACKING MATERIALS AND CHEMISTRIES

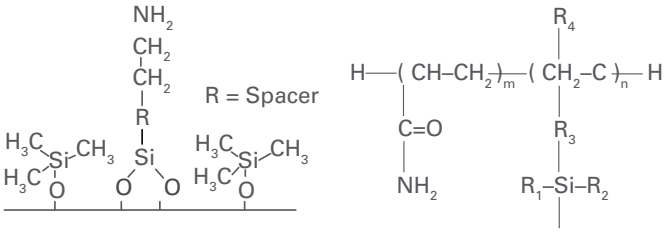
TSKgel Amide-80 offers an excellent alternative to amino-bonded stationary phases and consists of 2, 3, 5 or 10 µm silica particles in a stainless steel format. Spherical silica particles are covalently bonded with carbamoyl groups (Figure 2). For years TSKgel Amide-80 columns have been the standard for the analysis of glycans in biopharma.

TSKgel Amide-80 2 µm UHPLC columns packed with 2 µm particles are the newest addition to the series. The 2 µm HILIC UHPLC columns can be used with HPLC and UHPLC systems. Hence, they support a smooth transfer of HILIC methods established on Amide-80 HPLC columns to UHPLC technology.

TSKgel NH_2-100 3 µm expands the selectivity range of TSKgel HILIC solutions by a very robust amino-phase. In contrast to conventional silica-based amino phases this column offers expanded stability under HILIC conditions. It is well suited for the analysis of all types of hydrophilic compounds. The NH_2-100 phase is based on a silica particle with 10 nm pore size, treated with a special endcapping procedure. Amino groups are introduced step wisely after endcapping (Figure 2).

FIGURE 2

STRUCTURES OF TSKgel HILIC PHASES



TSKgel NH_2-100
*The spacer (R) contains secondary as well as tertiary amino groups.

TSKgel Amide-80

FEATURES OF TSKgel HILIC COLUMNS

TSKgel Amide-80

The bonded phase does not react with reducing sugars. Anomer formation can be prevented by raising mobile phase temperature up to 50 °C for 2 & 3 µm columns and up to 80 °C for 5 & 10 µm columns.

Stable in 100% organic for normal phase applications

Can be used with all kinds of detectors including evaporative light scattering (ELS) and mass spec (MS) detectors

Applications:
saccharides and Oligosaccharides
Polyols (polyalcohols)
Polar drugs and drug metabolites
peptides
Water-soluble vitamins
Melamine and cyanuric acids
oligonucleotides
Nucleobases

TSKgel NH_2-100

The bonded phase is more stable than conventional amino phases due to a special endcapping prior to introduction of aminoalkyl groups. Amino-bonded phases can react with a reducing sugar to form a Schiff base

Stable in 100% organic for normal phase applications

Can be used with all kinds of detectors including evaporative light scattering (ELS) and mass spec (MS) detectors

Applications:
Saccharides and Oligosaccharides
Polyols (polyalcohols)
Polar drugs and drug metabolites
Methotrexate polyglutamate derivatives
Water-soluble vitamins
Nucleic acid fragments
Pyridylaminated oligosaccharides

HILIC
ABOUT TSKgel AMIDE-80

- Optimized for efficient glycosylation analysis
- Available in 2, 3, 5 and 10 µm particle size
- UHPLC columns (2 µm) support easy method transfer from HPLC to UHPLC
- High speed and high resolution analysis with HPLC and UHPLC systems
- Ideal for mass spectrometric detection

The amide stationary phase provides a unique selectivity under regular normal phase conditions or in the hydrophilic interaction (HILIC) mode. Amide-80 shows higher retention of polar compounds than other amide phases.

TSKgel Amide-80 columns packed with 2 µm silica based particles are the latest additions to the well-known TSKgel Amide-80 series. They are especially suited for use in UHPLC systems, as the reduced system volume and optimized detector specifications of UHPLC systems help to maintain the high resolution that can be achieved with 2 micron stationary phases.

Figure 3 shows the characterization of the new 2 µm version of TSKgel Amide-80 compared to the renowned 3 µm Amide-80 based on the system proposed by Y. Kawachi et al. (J. Chromatogr. A, 1218 (2011) 5903 ff).

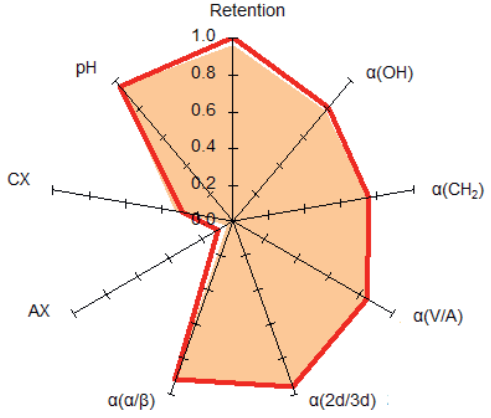
TSKgel Amide-80 can be operated over a temperature range of 10-80 °C (10-50 °C for Amide-80 2 & 3 µm). In general, retention times for carbohydrates decrease with increasing temperature. Below certain temperatures some carbohydrates may elute as split peaks. In this case, column heating or addition of triethylamine to the mobile phase is required. The pH range of mobile phase for TSKgel Amide-80 is 2.0-7.5 with a maximum salt concentration of 100 mmol/L. TSKgel Amide-80 is stable in 100% organic for normal phase separations; however, in HILIC mode the addition of water is necessary to create the water-rich surface layer.

DURABILITY

The high stability of TSKgel Amide-80 columns is demonstrated in Figure 4 showing the same analysis on a 3 µm Amide-80 column 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

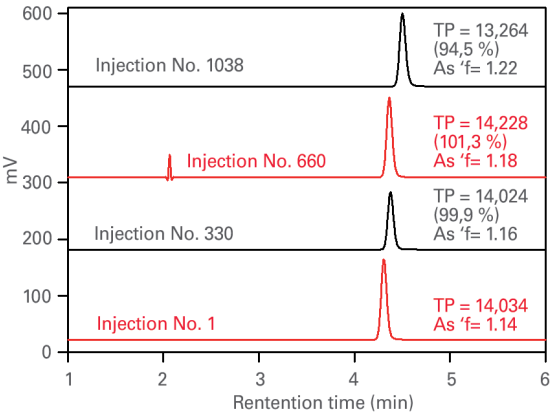
TSKgel AMIDE-80 SELECTIVITY



Columns: TSKgel Amide-80 2 µm, 4.6 mm ID x 15 cm L, solid line
TSKgel Amide-80 3 µm, 4.6 mm ID x 15 cm L, colored area

FIGURE 4

DURABILITY OF TSKgel AMIDE-80 3 µm



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

HILIC
TSKgel AMIDE-80 UHPLC APPLICATIONS

HIGH SPEED UHPLC ANALYSIS

The reduced particle size of the TSKgel Amide-80 2 µm column considerably increases theoretical plates and resolution. The high resolution can be exploited to drastically reduce analysis time. Figure 5 shows an almost 10 fold reduction in total analysis time, while resolution is only reduced by about 40 percent when using a 5 cm short TSkgel Amide-80 2 µm column and increased flow rate compared to the 3 µm column with 15 cm length and standard flow rate. Despite the relatively high flow rate, the pressure drop is moderate (< 20 MPa). This allows the use of a HPLC system, even though any system used with small particle columns should be optimized with regard to void volume, detector cell and detection parameters.

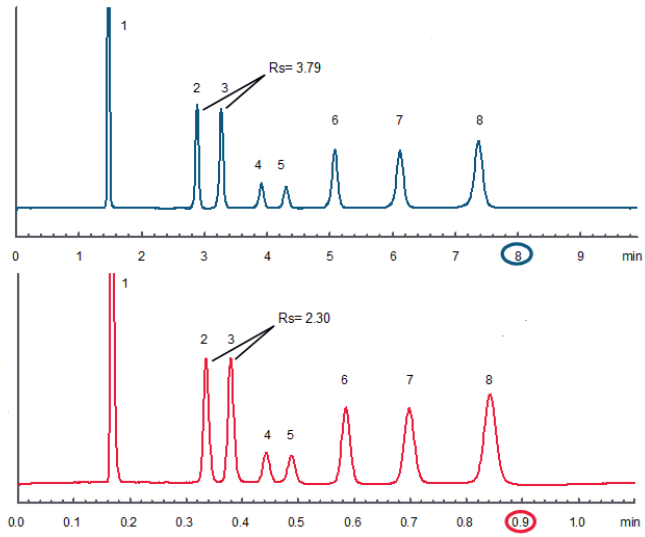
UHPLC GLYCOSYLATION ANALYSIS

TSKgel Amide-80 2 µm provides the same unique selectivity as TSKgel Amide-80 3 µm or 5 µm that are applied for glycan analysis in many QC labs for years. The suitability of the 2 micron material for glycosylation analysis of labelled glycans with fluorescence detection is shown in Figure 6. Several peaks of pyridylaminated glycans were separated for both mouse IgG and human IgG. These peaks were similar in elution time to 6-8 mer glucose.

Pyridylamination is a fluorescence-tagging method for oligosaccharides that enables measurement and structural analyses of glycans.

FIGURE 5

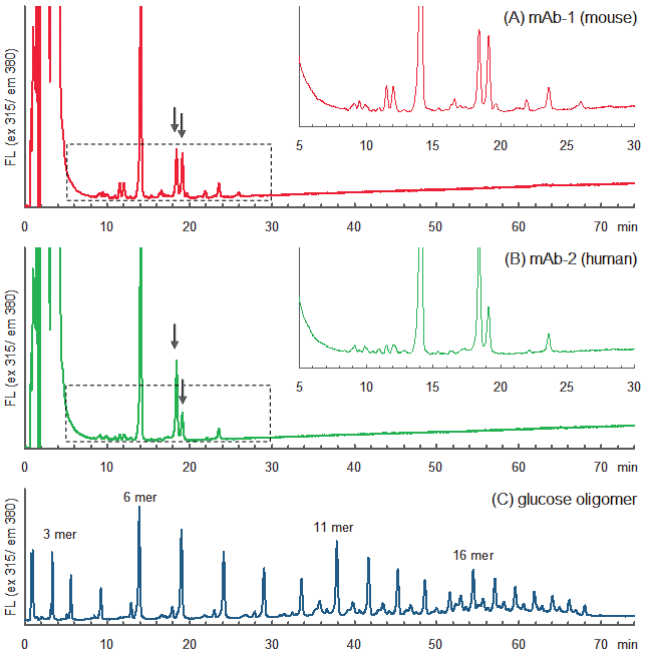
ULTRA-FAST HILIC ANALYSIS



(A) Column:	TSKgel Amide-80 2 µm, 3.0 mm ID x 5 cm L, red
Flow rate:	1.29 mL/min
(B) Column:	TSKgel Amide-80 3 µm, 3.0 mm ID x 15 cm L, blue
Flow rate:	0.43 mL/min
Mobile phase:	20 mmol/L NH_4OAc (pH 4.7) / acetonitrile = 10 / 90
Temperature:	40 °C
Detection:	UV @ 254 nm
Injection vol.:	2 µL
Samples:	1. toluene (1 g/L)
	2. theophylline (0.1 g/L)
	3. theobromine (0.1 g/L)
	4. NPβb-Glu (0.1 g/L)
	5. NPαa-Glu (0.1 g/L)
	6. 2'-deoxyuridine (0.1 g/L)
	7. 5-methyluridine (0.1 g/L)
	8. uridine (0.1 g/L)

FIGURE 6

SEPARATION OF FAB AND FC FRAGMENTS



Column:	TSKgel Amide-80 2 µm, 2.0 mm ID x 15 cm L
Mobile phase:	A: 200 mmol/L acetic acid + triethylamine, pH 7.3
	B: acetonitrile
Gradient:	75% B (0-5 min), 75-50% B (5-80 min, linear)
Flow rate:	0.5 mL/min
Temperature:	40 °C
Detection:	fluorescence (EX @ 315 nm, EM @ 380 nm)
Injection vol.:	50 µL
Sample:	(A) Pyridylaminated oligosaccharides released from mAb-1 (mouse)
	(B) Pyridylaminated oligosaccharides released from mAb-2 (human)
	(C) PA-glucose ladder (3-22 mer) (TaKaRa Bio)

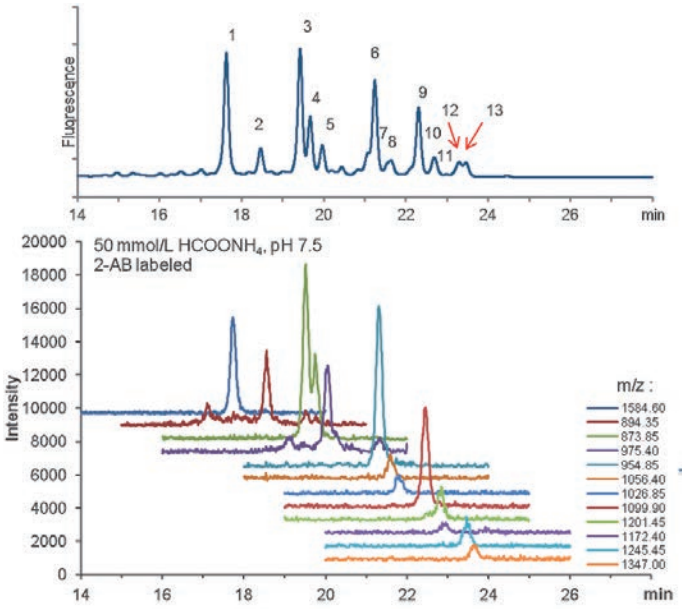
HILIC
TSKgel AMIDE-80 UHPLC-MS APPLICATIONS

UHPLC-MS ANALYSIS OF 2-AB LABELLED N-GLYCANS

Figure 7 shows the UHPLC analysis of 2-aminobenzamide (2-AB) labelled glycans with mass spectrometric detection on a TSKgel Amide-80 2 µm. 2-aminobenzamide is one of the most common labels used for glycosylation analysis.

FIGURE 7

UHPLC-MS ANALYSIS OF 2-AB GLYCANS ON TSKgel AMIDE-80 2 µM



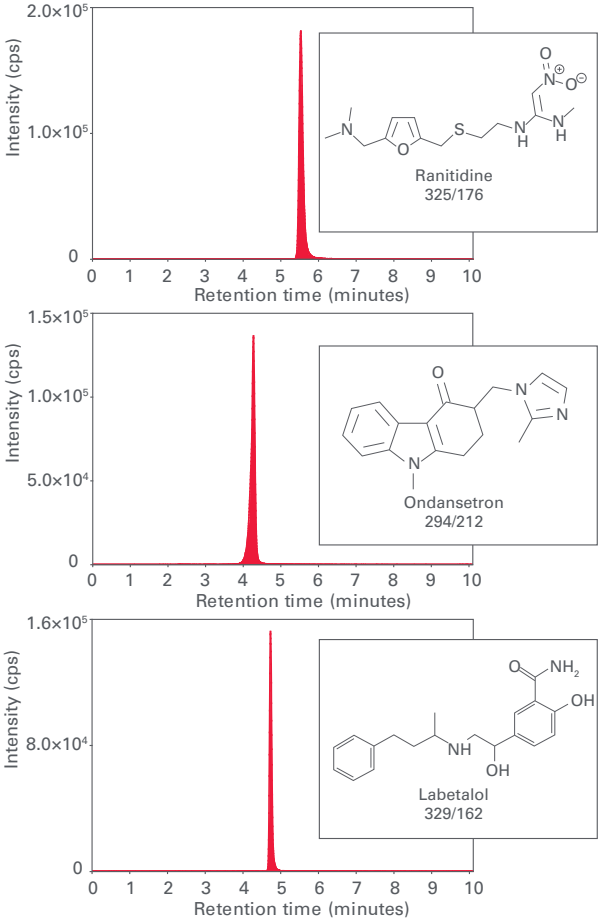
Column:	TSKgel Amide-80 2 µm, 2.0 mm ID x 15 cm L
Mobile phase:	A: 50 mmol/L $HCOONH_4$, pH 7.5
	B: acetonitrile
Gradient:	75 %B (0-5 min), 75-50% B (5-30 min, linear)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	(a) fluorescence (EX @ 315 nm, EM @ 380 nm)
	(b) LC/MS, ESI positive, SIM (Shimadzu LCMS-8030)
Injection vol.:	50 µL
Sample:	2-AB labelled N-glycans released from human IgG (Ludger, cat.# CLIBN-IGG-01)

HILIC-MS ANALYSIS OF POLAR DRUGS

TSKgel Amide-80 columns are also a valuable tool for the analysis of small molar mass polar drugs that are not sufficiently retained on reversed phase columns. Figure 8 shows the separation of polar drug standards in HILIC mode using a 3 µm TSKgel Amide-80 column coupled with electrospray ionization mass spectroscopy (ESI/MS). Due to the high organic content of the eluent, HILIC analysis provides increased detection sensitivity.

FIGURE 8

SEPARATION OF POLAR DRUG STANDARDS



Column:	TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm L
Mobile phase :	A: 10 mol/L ammonium formate, pH 3.75
	B: ACN
Gradient :	0 min (90% B) 10 min (40% B) 13 min (40% B)
Flow rate :	0.2 mL/min
Injection vol.:	5 µL (50 µg/L)
Samples:	ranitidine
	ondansetron
	labetalol
Instrument :	Q TRAP (AB Sciex) LC/MS/MS
Ion Source:	ESI+

HILIC
TSKgel AMIDE-80 APPLICATIONS

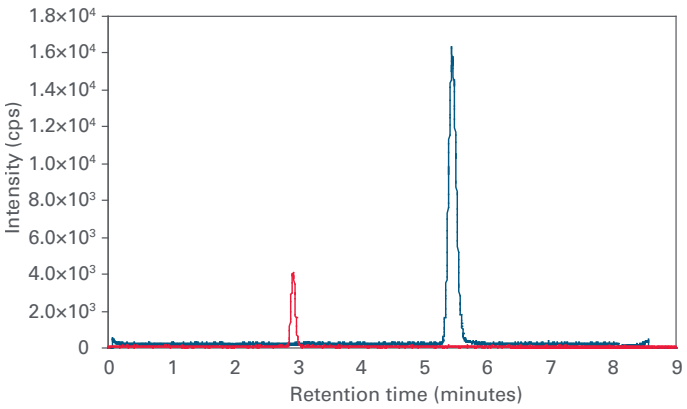
MELAMINE AND CYANURIC ACID IN MILK

Tosoh scientists developed a method for the simultaneous determination of melamine and cyanuric acid in milk by HILIC/MS/MS using a 3 µm TSKgel Amide-80 column. Milk was spiked with melamine and cyanuric acid standards to serve as a model sample. High recovery and excellent resolution was obtained for both compounds, as shown in Figure 9.

Multiple Reaction Monitoring is a mode of MS/MS that yields maximum sensitivity and selectivity for known target analytes. Figure 10 shows the results of this type of mass analysis on unspiked and spiked milk samples. The figure demonstrates that the original milk sample did not contain any amount of either melamine or cyanuric acid. After adding the compounds to the milk sample, melamine and cyanuric acid were independently detected, with more than sufficient resolution between the compounds.

FIGURE 9

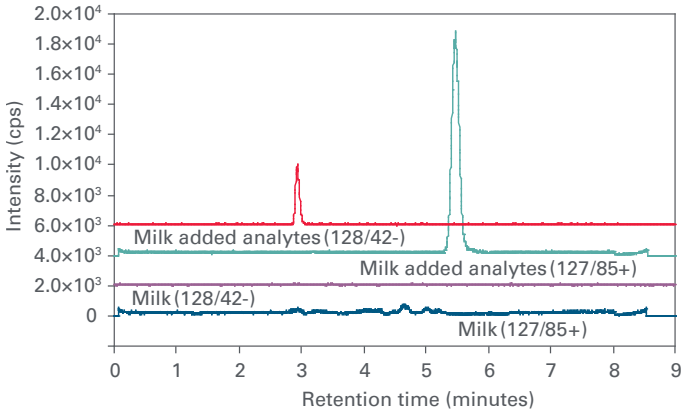
SEPARATION OF MELAMINE AND CYANURIC ACID IN MILK



Column:	TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm L
Mobile phase:	A: 0.05% formic acid in H_2O
	B: 0.05% formic acid in ACN
	A/B = 25/75
Flow rate:	0.2 mL/min
Temperature:	40 °C
Injection vol.:	5 µL
Instrument:	Q TRAP® (AB Sciex)
Ion source:	ESI
	127/85+ (melamine)
	128/42- (cyanuric acid)

FIGURE 10

MULTIPLE REACTION MONITORING (MRM) CHROMATOGRAMS OF MILK AND SPIKED MILK SAMPLES - 10 PPB EACH



Column:	TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm L
Mobile phase:	A: 0.05% formic acid in H2O
	B: 0.05% formic acid in ACN
	A/B = 25/75
Flow rate:	0.2 mL/min
Temperature:	40 °C
Injection vol.:	5 µL
Instrument:	Q TRAP (AB Sciex)
Ion source:	ESI
	127/85+ (melamine)
	128/42- (cyanuric acid)

HILIC
ABOUT TSKgel NH2-100

- Alternative HILIC selectivity
- Better stability than conventional amino phases
- Novel bonding chemistry

TSKgel NH_2-100 amino columns expand the range of TSKgel columns for hydrophilic interaction liquid chromatography. Offering a different selectivity from the well-known TSKgel Amide-80 series, these amino-bonded phase columns stand out by providing much improved chemical stability than conventional amino phases. Due to a high ligand density and large surface area, these columns show stronger retention of polar compounds than TSKgel Amide-80.

TSKgel NH_2-100 columns are packed with 3 µm silica particles. A novel bonding strategy was adopted to improve chemical stability. First, the silica is encapped with a trimethylsilane reagent. The resulting bonded phase provides a better safeguard against hydrolysis of the underlying silica.

TSKgel NH_2-100 columns are unique in that the ligand not only has a terminal primary amino group as expected, but that the spacer also incorporates secondary as well as tertiary amino groups. Anionic compounds are retained on the column by ionic interaction. This allows for the use of salt gradients in addition to acetonitrile gradients. Thus, the columns can be used as mixed mode columns under some conditions.

Also available within this line is a TSKgel NH_2-100 DC column that connects directly to TSKgel reversed phase columns. The DC in the name emphasizes this Direct Connect aspect. This column shows high retention for hydrophilic compounds/ions. A male outlet fitting enables the direct connection to the female end-fitting of a TSKgel reversed phase column. This allows for the simultaneous separation of an active pharmaceutical ingredient (API) and its counterion without the loss of column efficiency experienced when connecting two columns with capillary tubing.

TSKgel NH_2-100 columns can be operated over a temperature range of 10-50 °C. In general, retention times for carbohydrates decrease with increasing temperature.

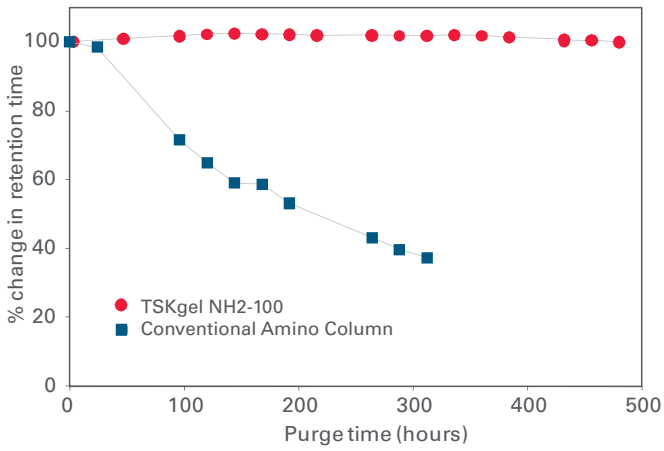
The mobile phase pH range for TSKgel NH_2-100 columns is 2.0 - 7.5 with a maximum salt concentration of 100 mmol/L. The columns are stable in 100% organic for normal phase separations; however, in HILIC mode a combination of aqueous and organic solvents is necessary to create the water-rich surface layer.

DURABILITY

Figure 11 shows the high stability of TSKgel NH_2-100 columns compared to a conventional amino phase. Both columns were purged for 300 hours in 25% water/75% acetonitrile and while the retention time of inositol on the conventional column decreased more than 60% from its initial retention time only a slight reduction is observed with the TSKgel NH_2-100 column after 400 hours.

FIGURE 11

CHEMICAL STABILITY STUDY



Columns:	TSKgel NH_2-100, 3 µm, 4.6 mm ID × 15 cm L
	Conventional Amino Column, 5 µm, 4.6 mm ID × 25 cm L
Mobile phase:	H_2O/ACN (25/75)
Flow Rate:	1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	inositol

HILIC
TSKgel NH2-100 APPLICATIONS

SEPARATION OF WATER-SOLUBLE VITAMINS

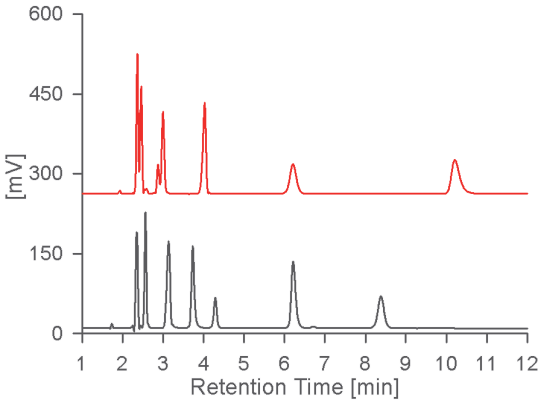
Figure 12 shows the separation of a standard solution of water soluble vitamins on a TSKgel NH_2-100 column compared to a TSKgel Amide-80 column. Dimension (4.6 mm ID x 15 cm L), particle size (3 µm), flow rate, and mobile phase were identical for both columns. The elution order of the compounds changes 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 13 compares the separation of methotrexate and its derivatives (MTXPG2~7) on TSKgel NH_2-100, 3 µm HILIC and TSKgel ODS-100V, 3 µm 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 12

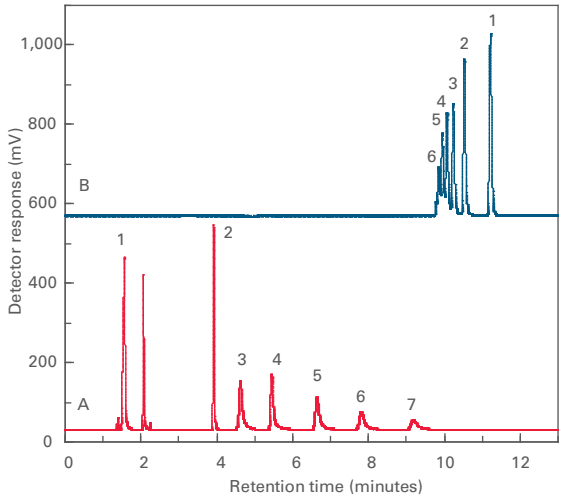
SEPARATION OF WATER SOLUBLE VITAMINS



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
Mobile phase:	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 vol.:	5 µL

FIGURE 13

SEPARATION OF METHOTREXATE AND DERIVATIVES



Columns:	A. TSKgel NH_2-100, 3 µm, 2.0 mm ID × 15 cm L B. TSKgel ODS-100V, 3 µm, 2.0 mm ID × 15 cm L
Mobile phase:	A: A) H_2O/ACN (10/90) + 0.1% TFA B) H_2O + 0.1% TFA B: A) H_2O/ACN (10/90) + 0.1% TFA B) ACN + 0.1% TFA
Gradient:	0 min (0%B) 15 min (40%B) 17 min (0%B)
Flow rate:	0.20 mL/min
Detection:	UV @ 313 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. MTX (MTXPG) 2. $MTXPG_2$ 3. $MTXPG_3$ 4. $MTXPG_4$ 5. $MTXPG_5$ 6. $MTXPG_6$ 7. $MTXPG_7$

HILIC
TSKgel NH2-100 APPLICATIONS

DRUG AND COUNTER ION ANALYSIS

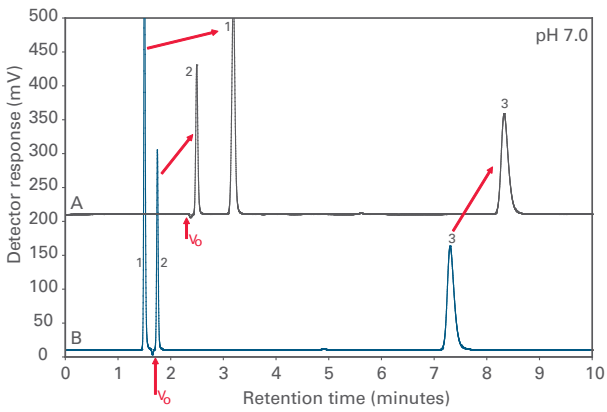
The TSKgel NH_2-100 DC column connects directly to TSKgel reversed phase columns and can be used to simultaneously analyze hydrophobic and hydrophilic/acidic compounds. Maleic acid and p-toluene sulfonic acid are commonly used as counter ions in pharmaceutical preparations. Both of these organic acids are hydrophilic and are not retained on a TSKgel ODS-100V reversed phase column at pH 7.0 in 70% methanol eluent (Figure 14B). 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 API desipramine becomes possible (Figure 14A). 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.

COLD MEDICINE INGREDIENTS

Guaiacol sulfonic acid, a hydrophilic counter ion, is an expectorant used in pharmaceutical cold preparations that are sold over the counter (OTC) in many countries. Guaiacol sulfonic acid elutes in the solvent front on a C18 column, but is retained on a TSKgel NH_2-100 DC, 3 µm column. Direct Connection (DC) of the TSKgel NH_2-100 DC, 3 µm column to a TSKgel ODS-100V, 3 µm column allows for the simultaneous determination of APIs and guaiacol sulfonic acid in a single run as shown in Figure 15.

FIGURE 14

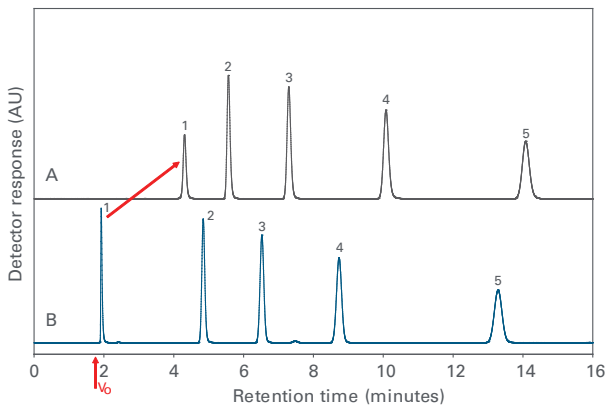
SIMULTANEOUS DETERMINATION OF MALEIC ACID AND THE API DESIPRAMINE AT PH 7.0



Columns:	A: TSKgel NH_2-100 DC, 3 µm, 4.6 mm ID × 5 cm L + TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm L B: TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm L
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)

FIGURE 15

SEPARATION OF COLD MEDICINE INGREDIENTS



Columns:	A) TSKgel NH_2-100 DC, 3 µm, 4.6 mm ID × 5 cm L + TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm L B) TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm L
Mobile phase:	50 mmol/L NaH_2PO_4, pH 2.5/MeOH = 65/35
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. guaiacol sulfonic acid (50 mg/L) 2. anhydrous caffeine (25 mg/L) 3. salicylamide (125 mg/L) 4. aspirin (250 mg/L) 5. ethenzamide (125 mg/L)

HILIC
ORDERING INFORMATION TSKgel HILIC

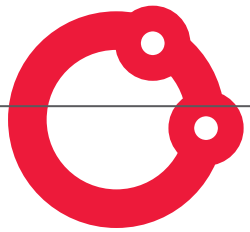
ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel HILIC Columns						
0021967	NH_2-100	2.0	5.0	3	≥ 4,000	15.0
0021968	NH_2-100	2.0	15.0	3	≥ 15,000	20.0
0021969	NH_2-100	4.6	5.0	3	≥ 6,000	5.0
0021970	NH_2-100	4.6	15.0	3	≥ 18,000	15.0
0021999	NH_2-100 DC	4.6	5.0	3	≥ 6,000	5.0
0023454	Amide-80	2.0	5.0	2	≥ 5,800	40.0
0023455	Amide-80	2.0	10.0	2	≥ 14,000	60.0
0023456	Amide-80	2.0	15.0	2	≥ 21,500	80.0
0023457	Amide-80	3.0	5.0	2	≥ 8,300	40.0
0023458	Amide-80	3.0	10.0	2	≥ 16,500	60.0
0023459	Amide-80	3.0	15.0	2	≥ 24,000	80.0
0021864	Amide-80	2.0	5.0	3	≥ 3,500	20.0
0021865	Amide-80	2.0	15.0	3	≥ 13,000	20.0
0022850	Amide-80	3.0	5.0	3		
0022851	Amide-80	3.0	10.0	3		
0022852	Amide-80	3.0	15.0	3		
0021866	Amide-80	4.6	5.0	3	≥ 6,000	20.0
0022849	Amide-80	4.6	10.0	3		
0021867	Amide-80	4.6	15.0	3	≥ 18,500	20.0
0020009	Amide-80	1.0	5.0	5	≥ 300	3.0
0020010	Amide-80	1.0	10.0	5	≥ 600	6.0
0021486	Amide-80	1.0	15.0	5	≥ 4,000	9.0
0021487	Amide-80	1.0	25.0	5	≥ 6,000	12.0
0019694	Amide-80	2.0	5.0	5	≥ 1,000	4.0
0019695	Amide-80	2.0	10.0	5	≥ 2,000	8.0
0019696	Amide-80	2.0	15.0	5	≥ 4,000	10.0
0019697	Amide-80	2.0	25.0	5	≥ 6,000	15.0
0021982	Amide-80 HR	4.6	25.0	5	≥ 18,000	15.0
0019532	Amide-80	4.6	5.0	5	≥ 1,500	5.0
0019533	Amide-80	4.6	10.0	5	≥ 3,000	5.0
0013071	Amide-80	4.6	25.0	5	≥ 8,000	15.0
0014459	Amide-80	7.8	30.0	10	≥ 5,000	7.0
0014460	Amide-80	21.5	30.0	10	≥ 8,000	3.0

HILIC
ORDERING INFORMATION TSKgel HILIC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	
Guardcolumns					
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
0023460	Amide-80 Guardcolumn (DC)	2.0	1.0	2	Direct connect guardcolumn
0021941	Amide-80 Guard cartridge, pk 3	2.0	1.0	5	For all 2 mm ID columns
0019010	Amide-80 Guard cartridge, pk 3	3.2	1.5	5	For all 4.6 mm ID columns
0019021	Amide-80 Guardcolumn	4.6	1.0	5	For all 4.6 mm ID columns
0014461	Amide-80 Guardcolumn	21.5	7.5	10	For 21.5 mm ID column
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
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



RPC
REVERSED PHASE CHROMATOGRAPHY

RPC PRODUCTS

RP COLUMNS FOR BIOMOLECULES

TSKgel Protein C_4-300
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
TSKgel Phenyl-5PW RP

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.

RPC HIGHLIGHTS

HIGHLIGHTS TSKgel Protein C4-300

- TSKgel Protein C4-300 is designed for reversed phase protein separations
- deal pore size for protein accessibility
- Thorough endcapping ensures low peak tailing
- High theoretical plate numbers through small particle size

HIGHLIGHTS TSKgel ODS-100

- General purpose reversed phase columns
- Two grades of hydrophobicity
- Proprietary endcapping for best-in-class surface properties

FEATURES

- Choice of C1 to C18 ligands
- Wide pore columns available
- Proprietary endcapping of residual silanol
- Available with silica or polymer matrix

BENEFITS

- Cover a wide spectrum of sample polarities
- deal for protein separations
- High column efficiencies
- No buffer pH restrictions

RPC
HOW DOES IT WORK?

Reversed Phase Chromatography (RPC) is one of the most frequently used chromatographic modes for analytical separations. Starting in the mid-1970s RPC has become the standard technique to analyze preferably small molecular weight compounds.

Reversed phase chromatography (RPC) retains molecules based on their hydrophobic character on a non-polar stationary phase. In an aqueous, moderately polar solvent the hydrophobic patches of the analyte molecule bind to an immobilized hydrophobic ligand. A mobile phase of increasing hydrophobicity (typically containing polar organic solvents such as methanol or acetonitrile) is used to release the bound molecule at a point at which the interaction between the exposed patches and the matrix is less favorable than the interaction between the molecule and the solvent. The molecule releases from the matrix and elutes. Elution can be performed either in isocratic or gradient mode. Isocratic elution is easy to realize, less expensive and allows solvent recycling. Gradient elution – the continuous reduction of polarity of the aqueous mobile phase by increasing percentage of organic solvent - delivers sharper peaks and faster separation.

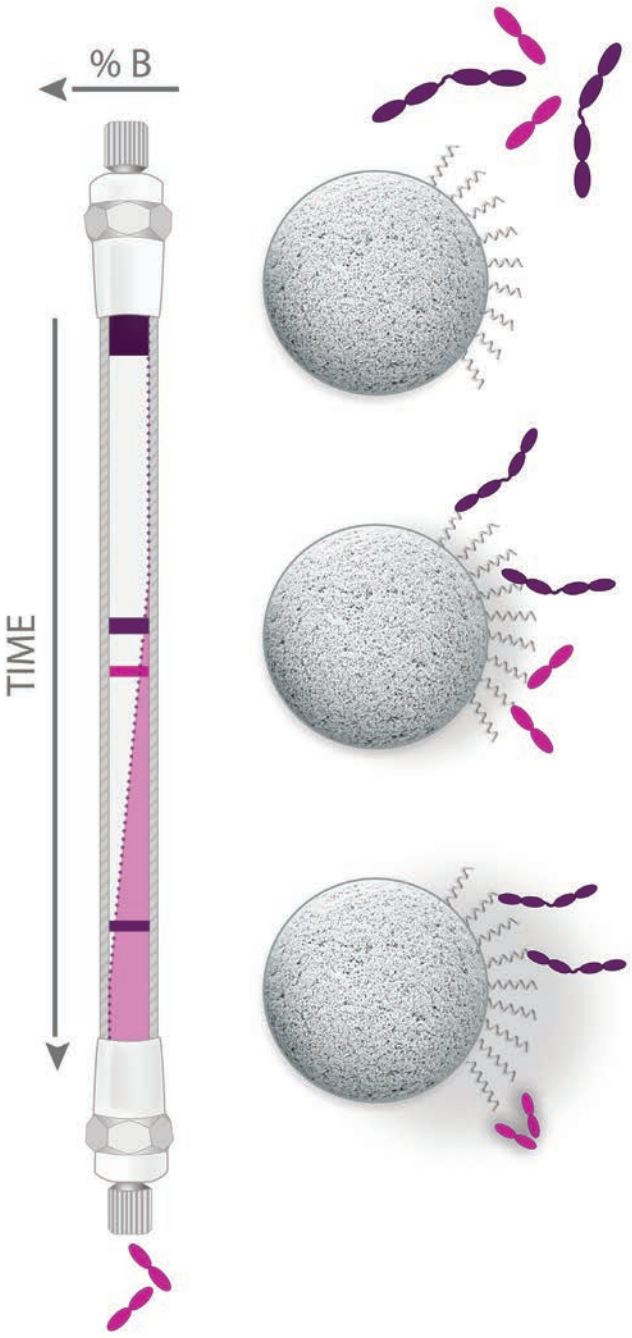
The binding of the analyte to the stationary phase is proportional to its hydrophobic surface area. Structural properties of the analyte therefore play an important role for reversed phase retention. Large hydrophobic surface areas increase retention whereas polar groups reduce retention. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased.

RP separation of peptides and proteins is usually performed by adding the volatile ionic modifier trifluoroacetic acid (TFA) to the mobile phase for ion pairing. Addition of TFA overcomes peak broadening and asymmetry (tailing) that are believed to result from interactions of peptides and proteins having a variety of polar, ionic, and hydrophobic sites with residual polar silica surfaces. For RP LC/MS analysis formic acid or ammonium formate are the most common modifiers.

RPC Applications range from small molecular weight compounds to biomolecules. RPC is also an 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 their elution.

FIGURE 1

REVERSED PHASE CHROMATOGRAPHY ILLUSTRATION



RPC STATIONARY PHASES

WHICH REVERSED PHASE COLUMN SHOULD I EVALUATE?

- Top performer for protein separation – TSKgel Protein C_4-300
- First choice for high throughput analysis – TSKgel ODS-140HTP
- Standard phases for small molecules – TSKgel ODS-100V/Z
- High pH analysis – TSKgel PW or NPR columns

PACKING MATERIALS AND CHEMISTRIES

The silica-based TSKgel RPC product line consists of two stationary phases with larger pore size designed for protein analysis (Protein C_4-300 and TMS-250) and several universal stationary phases designed for the analysis of low molar mass compounds, including active pharmaceutical ingredients (API), derivatized amino acids, steroids, lipids, fatty acids, etc.

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 silica stationary phases are subsequently endcapped by derivatization with trimethylsilyl groups to deactivates residual silanol groups.

Polymer-based reversed phase columns (Polymethacrylate) 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. This 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.

Tosoh Bioscience offers analytical and semi preparative reversed phase (RP) HPLC columns packed with silica or polymer based porous or non-porous beads. They are well suited for a broad range of applications.

Silica-based Columns	Polymer-Based columns
High purity type B silica High efficiencies Excellent recoveries Low bleed for MS	Hydrophilic backbone to improve recovery and reduce secondary interactions. pH stable from 1 to 12. Compatibility with organic solvents eliminates swelling
An excellent choice for analysis of small molecules and peptides. Grouped into six product families	An excellent choice for large MW biomolecules (>1.0 × 10^4 Da) and for analyzing small MM compounds at high pH. Offered in 4 different chemistries.
Protein C_4-300 ODS-100V and 100Z (10 nm) ODS-140HTP SuperSeries High efficiency (14 nm) Speciality silica columns	• Octadecyl-2PW (12.5 nm) • Octadecyl-4PW (50 nm) • Phenyl-5PW RP (100 nm) • Octadecyl-NPR (non-porous)

RPC COLUMN SELECTION

Properties of Silica-Based TSKgel RPC Columns

Column	Functional group	End-capped	% Carbon	Particle size (µm)	Pore size (nm)	Application/Features
Protein C_4-300	C4 alkyl, polymeric	Yes	3	3	30	For recovery and resolution of large biomolecules, such as proteins
ODS-140HTP	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC applicable; high throughput separations; high resolution and short analysis time at moderate pressures
ODS-100V	C18 alkyl, monomeric	Yes	15	3, 5	10	Initial choice; general purpose column
ODS-100Z	C18 alkyl, monomeric	Yes	20	3, 5	10	Initial choice; general purpose column
ODS-120T	C18 alkyl, polymeric	Yes	22	5, 10	15	Specialty column for analysis of peptides, small proteins, and small molecular weight compounds
ODS-120A	C18 alkyl, polymeric	No	22	5, 10	15	Specialty column for analysis of polyaromatic hydrocarbons. Best choice for steric selectivity
ODS-80TS	C18 alkyl, monomeric	Yes	15	5, 10	8	Low MW pharmaceuticals, bases, nucleosides and nucleotides. Ideal for strongly basic or charged compounds
ODS-80TS QA	C18 alkyl, monomeric	Yes	15	5	8	Tighter specs than standard ODS-80Ts
ODS-80TM	C18 alkyl, monomeric	Yes	15	5, 10	8	General purpose column for low MW pharmaceuticals, bases, nucleosides and nucleotides
Oligo-DNA RP	C18 alkyl, monomeric	No	10	5	25	For analysis and purification of oligonucleotides, RNA and DNA-fragments
Octyl-80TS	C8 alkyl, monomeric	Yes	10	5	8	deal choice for highly hydrophobic small molecules; reduced tailing when analyzing basic compounds
Super-ODS	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC-like resolution and speed with conventional HPLC systems; improved sensitivity; savings in time and solvent; less hydrophobic than C18; allows for rapid, high resolution separations of small proteins, pharmaceuticals, and aromatic compounds
Super-Octyl	C8 alkyl, polymeric	Yes	5	2.3	14	
Super-Phenyl	Phenyl alkyl, polymeric	Yes	3	2.3	14	
CN-80TS	CN, monomeric	Yes	9	5	8	Polar peptides, amino acids, and other pharmaceutical and food & beverage products
TMS-250	C1 alkyl, monomeric	Yes	5	10	25	For recovery and resolution of large biomolecules, such as proteins

Properties of Polymer-Based TSKgel RPC Columns

Column	Functional group	End-capped	% Carbon	Particle size (µm)	Pore size (nm)	Application/Features
Octadecyl-2PW	C18 alkyl, monomeric	-	-	5	12.5	Peptides up to 8,000 Da and small proteins
Octadecyl-4PW	C18 alkyl, monomeric	-	-	7, 13	50	Great for high pH separations of small molecules and proteins; Available in analytical and semi-preparative scale
Phenyl-5PW RP	Phenyl, monomeric	-	-	10, 13	100	deal for large, globular protein samples up to 1.0×10^6 Da; highly stable in low and high pH environments
Octadecyl-NPR	C18 alkyl, monomeric	-	-	2.5	non-porous	High efficiency separations and fast analysis of peptides and proteins with excellent pH stability

RPC - BIOMOLECULES
ABOUT TSKgel PROTEIN C4-300

- Optimized pore size for efficient analysis of proteins
- Endcapping ensures good peak shapes
- Small particle size for high theoretical plate numbers

TSKgel Protein C4-300 PROPERTIES

Silica based 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 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.

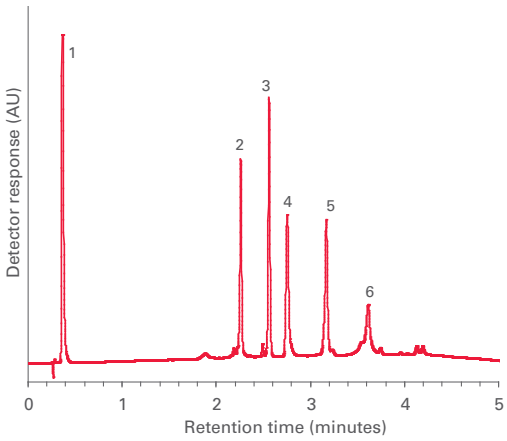
TSKgel Protein C4-300 APPLICATIONS

Fast Protein Separation

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 1). 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.

FIGURE 1

HIGH SPEED SEPARATION OF PROTEINS



Column:	TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 5 cm L
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 C4 RPC Columns for Protein Analysis						
0022827	TSKgel Protein C4-300	4.6	5.0	3	> 6,000	10
0022828	TSKgel Protein C4-300	4.6	10.0	3	> 11,500	17.5
0022829	TSKgel Protein C4-300	4.6	15.0	3	> 17,000	25
0022830	TSKgel Protein C4-300	2.0	5.0	3	> 4,500	15
0022831	TSKgel Protein C4-300	2.0	10.0	3	> 10,000	22.5
0022832	TSKgel Protein C4-300	2.0	15.0	3	> 15,500	30
Guardcolumns						
0022833	Protein C4-300 Guard Cartridge, 3 p	3.2	1.5	For all 4.6 mm ID Protein C4-300 columns		
0022834	Protein C4-300 Guard Cartridge, 3 p	2.0	1.0	For all 2 mm ID Protein C4-300 columns		
0019018	Cartridge holder			For 3.2 mm ID cartridges		
0019308	Cartridge holder			For all 2 mm ID Guardcolumns		

RPC - BIOMOLECULES
ABOUT TSKgel TMS-250 /OligoDNA RP

- Large 25 nm pore size base silica suited for biopolymers
- C1 low hydrophobicity functional group for protein analysis in TMS-250
- C18 bonded phase optimized for oligonucleotide analysis in OligoDNA RP

TSKgel TMS-250 PROPERTIES AND APPLICATIONS

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. On TSKgel TMS-250 proteins show sharper peaks than on ohter wide-pore C8 or C18 columns. It can accommodate even large proteins, such as aldolase (158 kDa). The good resolution of proteins on TSKgel TMS-250 columns is shown in Figure 2.

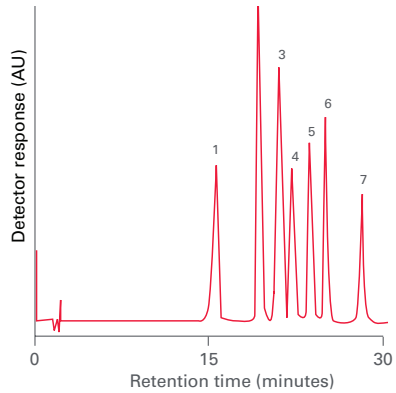
TSKgel OligoDNA PROPERTIES AND APPLICATIONS

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.

Figure 3 shows 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. The purity of the isolated oligonucleotide was subsequently verified on an analytical 4.6 mm ID TSKgel OligoDNA-RP column.

FIGURE 2

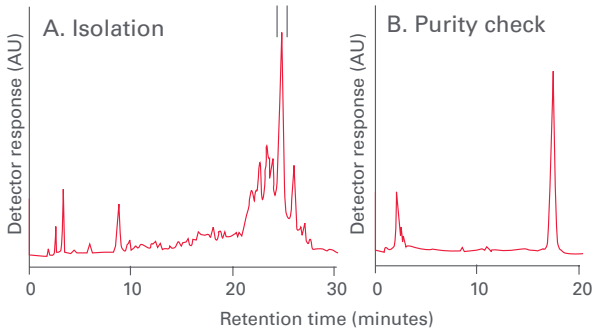
HIGH RESOLUTION PROTEIN SEPARATION ON TSKgel TMS-250



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
Mobile phase: 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 3

PURIFICATION AND ANALYSIS OF 49-MER OLIGONUCLEOTIDE



Columns: A. TSKgel OligoDNA-RP, 5 µm, 7.8 mm ID × 15 cm L
B. TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 15 cm L
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 nm
Sample: synthetic 49-mer oligonucleotide,
d(AGCTTGGGCTGCAGGTCGTCTCTAGAGGATCCCCGGGCGAGCTCGAATT)

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
Special TSKgel RPC Columns						
0013352	OligoDNA RP	4.6	15.0	5	7,000	12.0
0013353	OligoDNA RP	7.8	15.0	5	7,000	12.0
0007190	TMS-250	4.6	7.5	10	1,500	2.0

RPC - UNIVERSAL
ABOUT TSKgel ODS-100V/Z

TSKgel ODS-100 is the first choice when a universal reversed phase column is needed

- Two levels of hydrophobicity (15% and 20 % carbon load)
- Small particle ultra-pure silica ensures high column efficiencies
- No non specific adsorption because of low residual silanol content

TSKgel ODS-100V AND ODS-100Z PROPERTIES

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. Table I summarizes the basic properties of ODS-100V and ODS-100Z stationary phases.

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.

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 I

PROPERTIES OF TSKgel ODS100V AND 100Z

	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 - UNIVERSAL
TSKgel ODS-100V/Z APPLICATIONS

COMPARISON OF SELECTIVITY WITH NIST STANDARD SRM 870

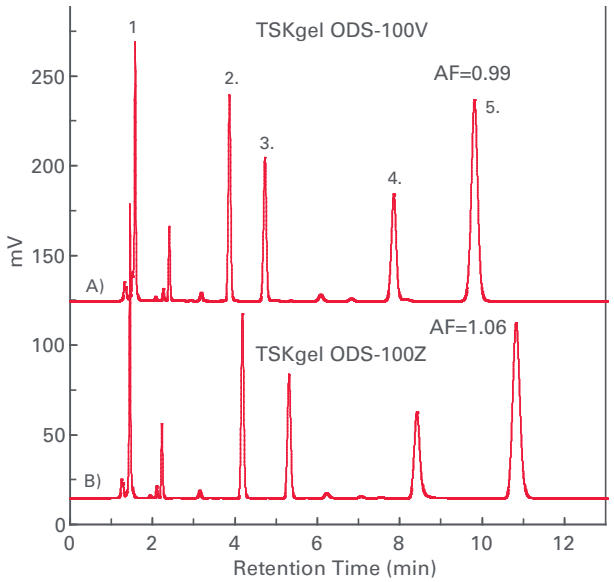
Standard Reference Material SRM 870 was developed by NIST (National Institute of Standards and Technology) as a means to classify the many commercially available reversed phase columns into closely-related groups. Amitriptyline, a tertiary amine, and quinizarin, a strong chelating compound, are included in the SRM 870 mixture, together with more traditional compounds. As shown in Figure 4, 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.

COMPARISON OF SELECTIVITY FOR 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 5. 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 4

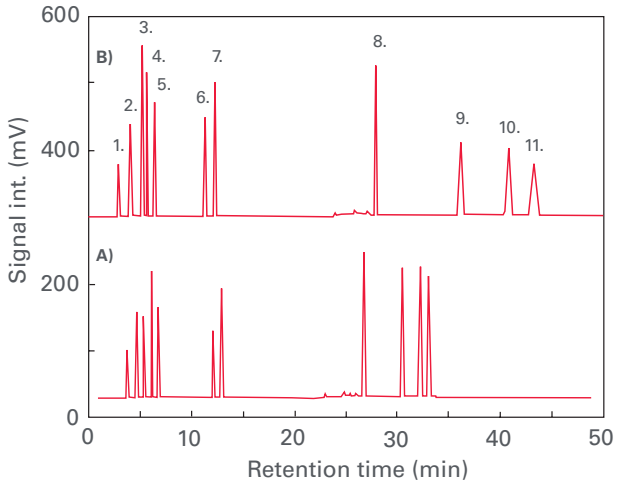
STANDARD REFERENCE MATERIAL SRM 879



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
Mobile phase:	20 mmol/L Phosphate buffer (pH 7.0)/MeOH (20/80)
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temp.:	40 °C
Injection vol.:	10 µL
Sample:	1. Uracil
	2. Toluene
	3. Ethyl benzene
	4. Quinizarin
	5. Amitriptyline

FIGURE 5

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
Mobile phase:	(A) 0.1% TFA in H2O; (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 @ 280 nm
Injection vol.:	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)

RPC - UNIVERSAL
TSKgel ODS-100V/Z APPLICATIONS

ORGANIC ACIDS

Organic acids play an important role in many metabolic processes, fermentation and food products. Figure 6 shows a baseline separation of 15 organic acids in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase.

POLYMER ADDITIVES

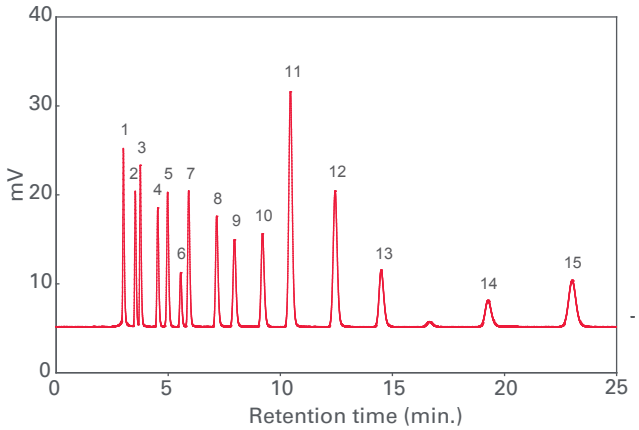
A baseline separation of 26 well known polymer additives is shown in Figure 7. Note that while a simple linear acetonitrile gradient was used, the column temperature was increased to 50 °C to achieve the required baseline separation on a TSKgel ODS-100V column.

NUCLEOTIDES

The analysis of mono-, di-, and tri-phosphorylated nucleotides on a TSKgel ODS-100V column is shown below (Figure 8). The separation is accomplished by adding a short chain ion pairing agent, t-butylamine, and adjusting the mobile phase pH to 6.8.

FIGURE 6

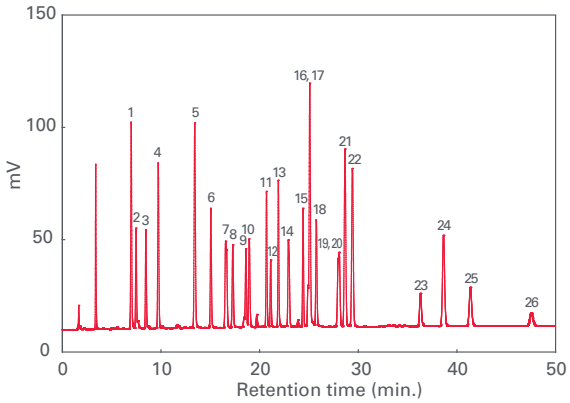
ANALYSIS OF ORGANIC ACIDS WITH TSKgel ODS-100V



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
Injection vol.:	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 7

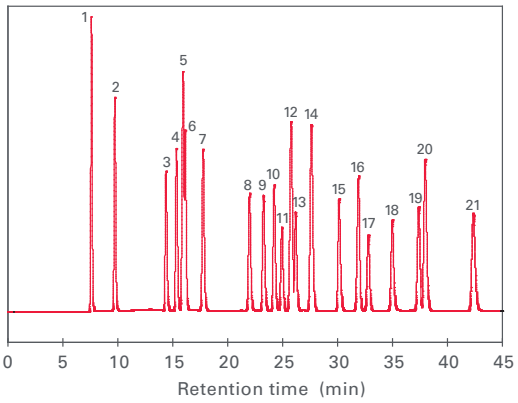
ANALYSIS OF POLYMER ADDITIVES WITH TSKgel ODS-100V



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 @ 225 nm
Injection vol.:	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 8

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 @ 260 nm
Injection vol.:	2 µL; 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 - UNIVERSAL
ORDERING INFORMATION TSKgel ODS-100V/Z

ORDERING INFORMATION

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

RPC - UNIVERSAL
ORDERING INFORMATION TSKgel ODS-100V/Z

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
0022721	ODS-100V	4.6	2.0	5	≥ 1,500	9.0
0022722	ODS-100V	4.6	3.5	5	≥ 3,000	9.0
0022723	ODS-100V	4.6	5.0	5	≥ 4,500	12.0
0022724	ODS-100V	4.6	7.5	5	≥ 7,000	18.0
0022725	ODS-100V	4.6	10.0	5	≥ 9,000	18.0
0021455	ODS-100V	4.6	15.0	5	≥ 14,000	18.0
0021456	ODS-100V	4.6	25.0	5	≥ 23,000	21.0
Guardcolumns for TSKgel ODS-100V Columns						
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	
0019018	Cartridge holder				For 3.2 mm ID cartridges	
0019308	Cartridge holder				For all 2 mm ID Guardcolumns	
TSKgel ODS-100Z 3 & 5 µm RPC C olumns						
0022726	ODS-100Z, pk 3*	2.0	1.0	3	≥ 500	30.0
0022727	ODS-100Z	2.0	2.0	3	≥ 1,500	12.0
0022728	ODS-100Z	2.0	3.5	3	≥ 4,000	15.0
0022729	ODS-100Z	2.0	5.0	3	≥ 5,700	15.0
0022730	ODS-100Z	2.0	7.5	3	≥ 8,600	21.0
0022731	ODS-100Z	2.0	10.0	3	≥ 11,500	24.0
0022732	ODS-100Z	2.0	15.0	3	≥ 17,500	24.0
0022733	ODS-100Z	2.0	25.0	3	≥ 28,000	30.0
0022734	ODS-100Z	3.0	2.0	3	≥ 2,000	12.0
0022735	ODS-100Z	3.0	3.5	3	≥ 4,000	12.0
0022736	ODS-100Z	3.0	5.0	3	≥ 6,000	15.0
0022737	ODS-100Z	3.0	7.5	3	≥ 9,000	21.0
0022738	ODS-100Z	3.0	10.0	3	≥ 12,000	24.0
0022739	ODS-100Z	3.0	15.0	3	≥ 18,000	24.0
0022740	ODS-100Z	3.0	25.0	3	≥ 29,000	30.0
0022741	ODS-100Z	4.6	2.0	3	≥ 2,500	12.0
0022742	ODS-100Z	4.6	3.5	3	≥ 4,500	12.0
0022743	ODS-100Z	4.6	5.0	3	≥ 6,500	15.0
0022744	ODS-100Z	4.6	7.5	3	≥ 9,750	21.0
0022745	ODS-100Z	4.6	10.0	3	≥ 13,500	24.0
0022746	ODS-100Z	4.6	15.0	3	≥ 19,500	24.0
0022747	ODS-100Z	4.6	25.0	3	≥ 30,000	30.0

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ORDERING INFORMATION TSKgel ODS-100V/Z

ORDERING INFORMATION

0022748	ODS-100Z, pk 3*	2.0	1.0	5	≥ 300	28.0
0022749	ODS-100Z	2.0	2.0	5	≥ 1,000	9.0
0022750	ODS-100Z	2.0	3.5	5	≥ 2,500	9.0
0021460	ODS-100Z	2.0	5.0	5	≥ 3,000	18.0
0022751	ODS-100Z	2.0	7.5	5	≥ 5,500	18.0
0022752	ODS-100Z	2.0	10.0	5	≥ 7,000	18.0
0021459	ODS-100Z	2.0	15.0	5	≥ 11,000	18.0
0022753	ODS-100Z	2.0	25.0	5	≥ 18,000	18.0
0022754	ODS-100Z	3.0	2.0	5	≥ 1,200	9.0
0022755	ODS-100Z	3.0	3.5	5	≥ 3,000	9.0
0022756	ODS-100Z	3.0	5.0	5	≥ 4,000	12.0
0022757	ODS-100Z	3.0	7.5	5	≥ 6,000	18.0
0022758	ODS-100Z	3.0	10.0	5	≥ 8,500	18.0
0022759	ODS-100Z	3.0	15.0	5	≥ 13,000	18.0
0022760	ODS-100Z	3.0	25.0	5	≥ 21,000	18.0
0022761	ODS-100Z	4.6	2.0	5	≥ 1,500	9.0
0022762	ODS-100Z	4.6	3.5	5	≥ 3,000	9.0
0022763	ODS-100Z	4.6	5.0	5	≥ 4,500	12.0
0022764	ODS-100Z	4.6	7.5	5	≥ 7,000	18.0
0022765	ODS-100Z	4.6	10.0	5	≥ 9,000	18.0
0021461	ODS-100Z	4.6	15.0	5	≥ 14,000	18.0
0021462	ODS-100Z	4.6	25.0	5	≥ 23,000	21.0

Guardcolumns for TSKgel ODS-100Z 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
0021454	ODS-100Z Guard Cartridge, pk 3*	3.2	1.5	5	For all ODS-100Z 4.6 mm ID columns
0019018	Cartridge holder				For 3.2 mm ID cartridges

**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 ANALYSIS ABOUT TSKgel ODS-140HTP

- Moderate pressure at high flow rates for HPLC and UHPLC use
- Small particle size for high resolution and high efficiency
- Moderate carbon content

TSKgel ODS-140HTP PROPERTIES

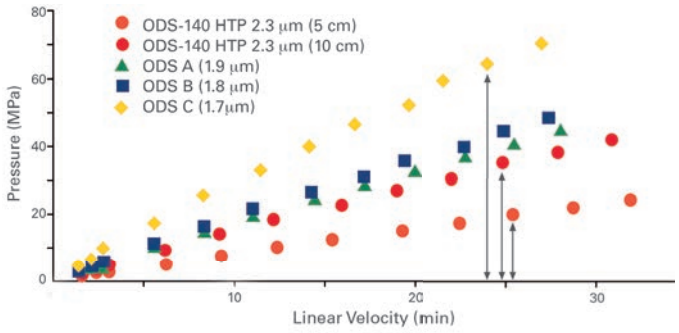
TSKgel ODS-140HTP columns were developed for use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations. They are packed with 2.3 µm particles, providing high resolution and short analysis times at moderate pressure. The lower pressure drop reduces 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 9).

TSKgel ODS-140HTP APPLICATIONS

Analysis of TCM components
n traditional Chinese medicine (TCM), 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 of components the column needs to provide a high peak capacity, as shown in Figure 10.

FIGURE 9

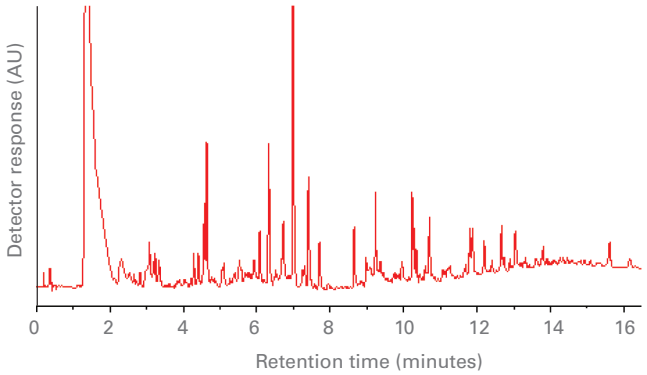
COLUMN BACKPRESSURE VERSUS PARTICLE SIZE



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
Mobile phase: H_2O/CH_2CN - 50/50

FIGURE 10

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
Mobile phase: 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 high thoughput ODS-140HTP 2.3 µm Columns							
0021927	TSKgel ODS-140HTP	2.1	5.0	2.3	14	≥ 7,000	60.0
0021928	TSKgel ODS-140HTP	2.1	10.0	2.3	14	≥ 14,000	60.0

RPC - FAST ANALYSIS
ABOUT TSKgel SUPER SERIES

TSKgel Super Series reversed phase columns are ideal for fast separations

- Three different hydrophobicities available (Phenyl, C8, C18)
- Monodisperse spherical 2.3 µm silica beads with 11 nm (110 Å) effective pore size
- Moderate pressure at high flow rates for HPLC and UHPLC use

TSKgel Super-ODS, Super-Octyl and Super-Phenyl phases 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 TSKgel Super-ODS, Super-Octyl and Super-Phenyl are 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 and ODS-140 HTP columns can be used on a regular HPLC system if the dead volume is minimized, although optimal results are obtained with an UHPLC system.

These recommendations are for 4.6 mm ID columns:.
Use proportionately lower values for 2 mm ID columns.
1. A guard filter is highly recommended .
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.
5. The smallest detector time constant should be selected.
6. The detector flow cell should be 2 µL or less.

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel Super RPC 2.3 µm Columns - silca based						
0020015	Super-ODS	1.0	5.0	2.3	≥ 1,500	15.0
0019541	Super-ODS	2.0	5.0	2.3	≥ 6,000	25.0
0019542	Super-ODS	2.0	10.0	2.3	≥ 12,000	25.0
0018154	Super-ODS	4.6	5.0	2.3	≥ 8,000	30.0
0018197	Super-ODS	4.6	10.0	2.3	≥ 16,000	30.0
0020013	Super-Octyl	2.0	5.0	2.3	≥ 1,500	15.0
0020014	Super-Octyl	2.0	10.0	2.3	≥ 5,000	30.0
0018275	Super-Octyl	4.6	5.0	2.3	≥ 8,000	30.0
0018276	Super-Octyl	4.6	10.0	2.3	≥ 16,000	30.0
0020017	Super-Phenyl	2.0	5.0	2.3	≥ 6,000	8.0
0020018	Super-Phenyl	2.0	10.0	2.3	≥ 12,000	15.0
0018277	Super-Phenyl	4.6	5.0	2.3	≥ 8,000	30.0
0018278	Super-Phenyl	4.6	10.0	2.3	≥ 16,000	30.0
Guardcolumn 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	

RPC - HIGH PH
ABOUT POLYMER BASED TSKgel RPC COLUMNS

Polymer based TSKgel columns allow operation at basic pH where silica-based columns have limited chemical stability.

- Chemically stable at pH 2-12, can be cleaned by using either strong acid or base
- Small, non-porous resin (NPR) particles feature fast kinetics
- Porous particles of various pore sizes to perfectly match sample molecular weight

TSKgel Octadecyl-NPR features a non-porous, small particle with a size of 2.5 µm and C18 chemistry. It provides a high column efficiency and quantitative protein recovery at sub-microgram loads. It can be used for small scale purification of proteins and peptides because it provides an improved recovery at low sample concentration over traditional porous resins.

TSKgel Octadecyl-2PW, the polymeric C18 phase with 5 µm particle size and 12.5 nm pores size can be used for analyzing small MW pharmaceutical compounds at basic pH. It provides faster analysis than competitive polymeric RPC columns.

TSKgel Octadecyl-4PW the polymeric C18 phase with 7 µm particle size and 50 nm pores size is recommended for peptides and small proteins.

TSKgel Phenyl-5PW RP has an average pore size of 100 nm and is ideal for the separation of large proteins. Due to its high capacity it is able to handle high loads. In comparison to the Phenyl-5PW packing material used in HIC, a higher C18 density (greater level of hydrophobicity) makes TSKgel Phenyl-5PW RP more suitable for use in RPC.

RPC - HIGH PH - ORDERING INFORMATION POLYMER BASED TSKgel RPC COLUMNS

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel RPC Columns - polymer based						
0018756	Phenyl-5PW RP	2.0	7.5	10	≥ 400	1.0
0008043	Phenyl-5PW RP	4.6	7.5	10	≥ 500	3.0
0016260	Phenyl-5PW RP	21.5	15.0	13	≥ 1,000	3.0
0014005	Octadecyl-NPR non-porous	4.6	3.5	2.5	≥ 1,000	20.0
0018754	Octadecyl-2PW	2.0	15.0	5	≥ 5,000	7.0
0017500	Octadecyl-2PW	4.6	15.0	5	≥ 6,000	10.0
0017501	Octadecyl-2PW	6.0	15.0	5	≥ 6,000	10.0
0018755	Octadecyl-4PW	2.0	15.0	7	≥ 2,000	10.0
0013351	Octadecyl-4PW	4.6	15.0	7	≥ 2,000	12.0
0016257	Octadecyl-4PW	21.5	15.0	13	≥ 2,000	2.5
TSKgel RPC Glass Columns - polymer based						
0014007	Phenyl-5PW RP Glass	8.0	7.5	10	≥ 700	2.0
Guardcolumns						
0019007	Phenyl-5PW RP Cartridge, pk 3 *	3.2	1.5	10	For P/N 0008043	
0017502	Octadecyl-2PW Guardcolumn	4.6	1.0	5	For P/N 0017500	
0017503	Octadecyl-2PW Guardcolumn	6.0	1.0	5	For P/N 0017501	
0019008	Octadecyl-4PW Cartridge, pk 3 *	3.2	1.5	7	For P/N 0013351	
Every Guardgel Kit contains Guardgel, Gelholder and Connector						
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	

RPC - TRADITIONAL - ABOUT TSKgel ODS-80Ts/Tm, OCTYL-80Ts, CN-80Ts

TSKgel ODS-80Ts/ODS-80Tm/Octyl-80Ts/CN-80Ts reversed phase columns are applied in several validated methods in pharmaceutical industry

- Three different hydrophobicities available (Cyano, C8, C18)
- Spherical silica with 8 nm (80 Å) pore size for fast mass transfer of small molecules
- High (Tm) or complete (Ts) endcapping shields the silica surface

TSKgel ODS-80Tm is a general purpose column for low MW pharmaceuticals, basic compounds, hydrophobic and hydrophilic peptides, nucleosides, nucleotides, purines and pyrimidines. TSKgel ODS-80Ts has a complete endcapping and is a good choice for strongly basic compounds and for applications that require operation at pH 7.5.

TSKgel Octyl-80Ts provides a faster kinetic than ODS, but lower hydrophobic selectivity TSKgel CN-80Ts is the 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	Maximum pressure drop (MPa)
TSKgel Standard RPC Columns						
0018150	ODS-80Ts	2.0	15.0	5	≥ 11,000	20.0
0018151	ODS-80Ts	2.0	25.0	5	≥ 18,000	30.0
0017200	ODS-80Ts	4.6	7.5	5	≥ 4,500	10.0
0017201	ODS-80Ts	4.6	15.0	5	≥ 11,000	20.0
0017202	ODS-80Ts	4.6	25.0	5	≥ 18,000	30.0
0017380	ODS-80Ts	21.5	30.0	10	≥ 6,000	6.0
0016651	ODS-80Tm	4.6	7.5	5	≥ 4,500	10.0
0008148	ODS-80Tm	4.6	15.0	5	≥ 11,000	20.0
0008149	ODS-80Tm	4.6	25.0	5	≥ 18,000	30.0
0014002	ODS-80Tm	21.5	30.0	10	≥ 6,000	6.0
0017344	Octyl-80Ts	4.6	15.0	5	≥ 11,000	20.0
0017345	Octyl-80Ts	4.6	25.0	5	≥ 18,000	30.0
0017348	CN-80Ts	4.6	15.0	5	≥ 11,000	20.0
0017349	CN-80Ts	4.6	25.0	5	≥ 18,000	30.0
Guardcolumns						
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	
0017385	ODS-80Ts Guardcolumn	21.5	7.5	10	For P/N 0017380	
0019004	ODS-80Tm Guard cartridge, pk 3 *	3.2	1.5	5	For 4.6 mm ID ODS-80Tm columns	
0014098	ODS-80Tm Guardcolumn	21.5	7.5	10	For P/N 0014002	
0019012	Octyl-80Ts Guard cartridge, pk 3 *	3.2	1.5	5	For all 4.6 mm ID ODS-80Ts columns	
0019013	CN-80Ts Guard cartridge, pk 3 *	3.2	1.5	5	For 4.6 mm ID CN-80Ts columns	
0019308	Guard cartridge holder	2.0	1.0		For all 2 mm ID cartridges	
0019018	Guard cartridge holder	3.2	1.5		For 3.2 mm ID cartridges	

RPC - TRADITIONAL
ABOUT TSKgel ODS-120

TSKgel ODS-120 are first generation reversed phase columns that are still applied in some validated methods in pharmaceutical industry

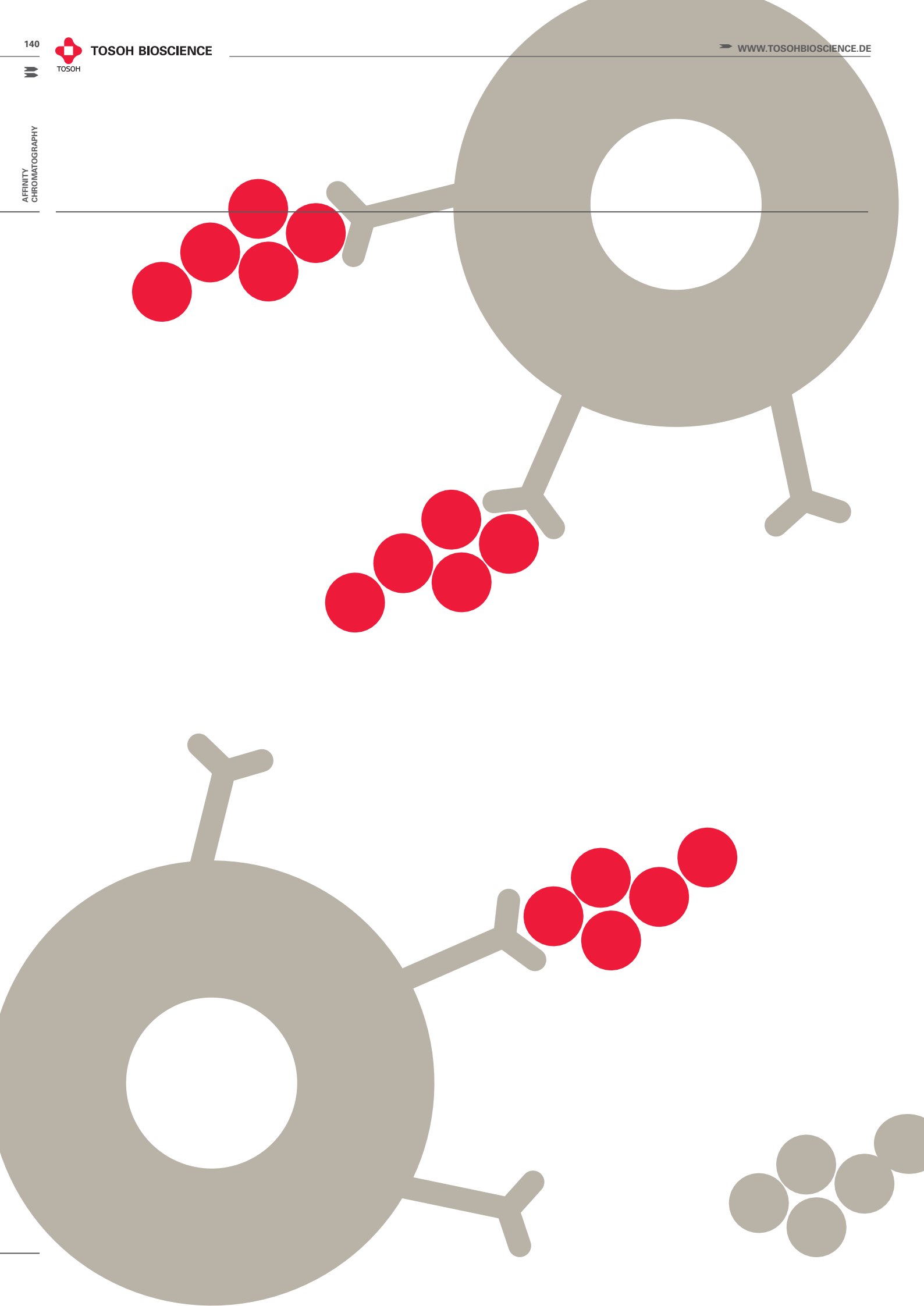
- Polymeric-bonded octadecyl (C18) groups improve peak shape for complex geometric isomers
- Available with (ODS-120A) or without endcapping (ODS 120T)

TSKgel ODS-120A and 120T provide a similar separation at low pH for a mixture of catecholamines, while at pH 6.0 the basic solutes interact with negatively charged residual silanol groups on ODS-120A, but not on the endcapped ODS-120T.

TSKgel ODS-120A exhibits improved peak shape for the separation of complex geometric isomers, such as poly-nuclear aromatic hydrocarbons (PAH) TSKgel 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	Maximum pressure drop (MPa)
TSKgel Standard RPC Columns						
0007636	ODS-120A	4.6	15.0	5	≥ 7,000	15.0
0007124	ODS-120A	4.6	25.0	5	≥ 10,000	20.0
0007129	ODS-120A	7.8	30.0	10	≥ 6,000	7.5
0006172	ODS-120A	21.5	30.0	10	≥ 6,000	6.0
0018152	ODS-120T	2.0	15.0	5	≥ 6,500	15.0
0018153	ODS-120T	2.0	25.0	5	≥ 10,000	20.0
0007637	ODS-120T	4.6	15.0	5	≥ 7,000	15.0
0007125	ODS-120T	4.6	25.0	5	≥ 10,000	20.0
0007130	ODS-120T	7.8	30.0	10	≥ 6,000	7.5
0007134	ODS-120T	21.5	30.0	10	≥ 6,000	6.0
Guardcolumns						
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 Guardcolumns	



ANTIBODY AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

FC RECEPTOR AFFINITY

TSKgel FcR-IIIA-NPR

PROTEIN A AFFINITY

TSKgel Protein A-5PW

Check out our clip on Protein A Affinity Chromatography:

youtu.be/pArLU69rHLM

ANTIBODY AFC HIGHLIGHTS

HIGHLIGHTS TSKgel FcR-IIIA-NPR

- Fast analysis of mAb ADCC activity and glycovariants
- Applicable in early R&D, upstream method development, and quality control
- Suitable for mAb analysis prior to or after purification
- Based on recombinant, human FcγRIIIa ligand

HIGHLIGHTS TSKgel Protein A-5PW

- Fast mAb titer determination for screening or upstream monitoring
- Applicable from initial R&D phase to process control
- Suitable for small scale antibody purifications
- Same ligand as TOYOPEARL Protein A-HC resin

FEATURES

- Recombinant affinity ligands
- Robust HPLC methods
- Optimized base particle/ligand design
- High reproducibility and long lifetime

BENEFITS

- Fast and robust antibody analysis
- Direct analysis of cell culture supernatant
- Applicable from early R&D screening to manufacturing
- Reduced costs per analysis

ANTIBODY AFC
FC RECEPTOR AFFINITY CHROMATOGRAPHY

HOW DOES IT WORK?

Fc γ-receptors (FcγRs) are found on the surface of effector cells in the immune system and play an important role in mediating cellular effector functions of antibody-based therapeutics through binding to the Fc-region of IgG. In Fc receptor affinity chromatography a recombinant Fc receptor ligand is immobilized on a stationary phase and utilized to analyze these effector functions.

Monoclonal antibodies (mAbs) continue to dominate the protein therapeutics market and antibody-dependent cell mediated cytotoxicity (ADCC) is one of their most important mechanisms of action (MOA) especially when applied in cancer therapy. ADCC begins when the Fab region of an antibody binds to an antigen on a target cell and the Fc domain binds Fcγ receptors on the surface of natural killer (NK) cells. Signaling through the Fcγ receptor triggers degranulation into a lytic synapse which ultimately leads to apoptosis (Figure 1). It is known that binding of the Fc part of antibodies to the Fcγ receptor is dependent on the glycan structures present at the Fc part of the IgG.

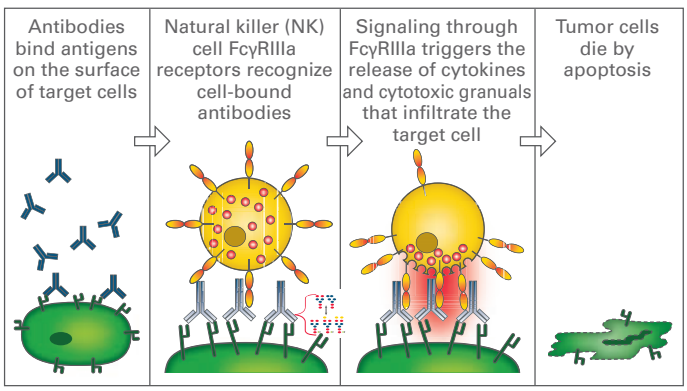
Current methods to determine ADCC activity are either based on bioassays or on surface plasmon resonance (SPR). Affinity chromatography with FcR ligands combines the high specificity of antibody-receptor-binding with the high reproducibility and easy handling of chromatographic techniques.

In FcγRIIIa chromatography, purified antibody or cell culture supernatant is injected under conditions that promote binding of mAbs to the FcγRIIIa phase. MAbs without ADCC activity will not bind to the receptor at all and elute in the void volume. Elution of bound, active mAb variants is performed by lowering the pH of the mobile phase in order to disrupt the target/ligand interactions. This typically results in three peaks representing mAb variants with high, medium, and low ADCC activity (Figure 2). As mentioned above, ADCC activity can be linked to the glycosylation of the antibody variants. It is known that ADCC activity is primarily modulated by the number of fucose and galactose units present in the glycan structure. While the presence of fucose units reduces ADCC activity, galactose units enhance it. Thus the high, medium, and low FcR affinity peaks also correlate with glycovariants of the antibody.

For ADCC related glycovariant analysis, the Fc receptor affinity chromatography can be an alternative to established methods by offering a simple and straightforward way for fast cell line screening in early development, cell culture condition optimization in upstream development, or batch analysis in production.

FIGURE 1

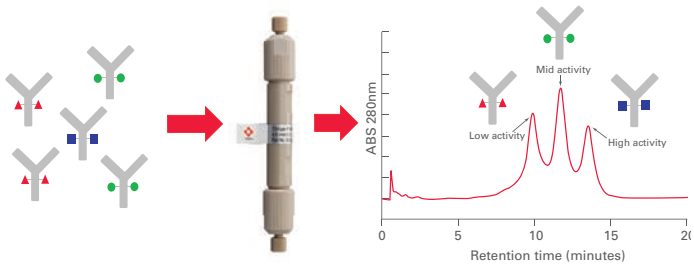
MECHANISM OF ANTIBODY-DEPENDENT CELL MEDIATED CYTOTOXICITY (ADCC)



Original image by Satchmo2000, distributed under a CC-BY 3.0 license.

FIGURE 2

SEPARATION OF mAb GLYCOFORMS ACCORDING TO THEIR AFFINITY TO FC RECEPTOR / ADCC ACTIVITY



ANTIBODY AFC
ABOUT TSKgel FcR-IIIA-NPR

TSKgel FcR-IIIA-NPR is specifically designed for fast determination of ADCC activity of monoclonal antibodies (mAbs) for

- Comparison between biosimilar/biobetter and originator product
- QC Analysis of lot-to-lot difference for therapeutic mAbs
- Monitoring fermentation stage of cell culture in upstream process
- Screening the potential of cell lines for ADCC activity

TSKgel FcR-IIIA-NPR PROPERTIES

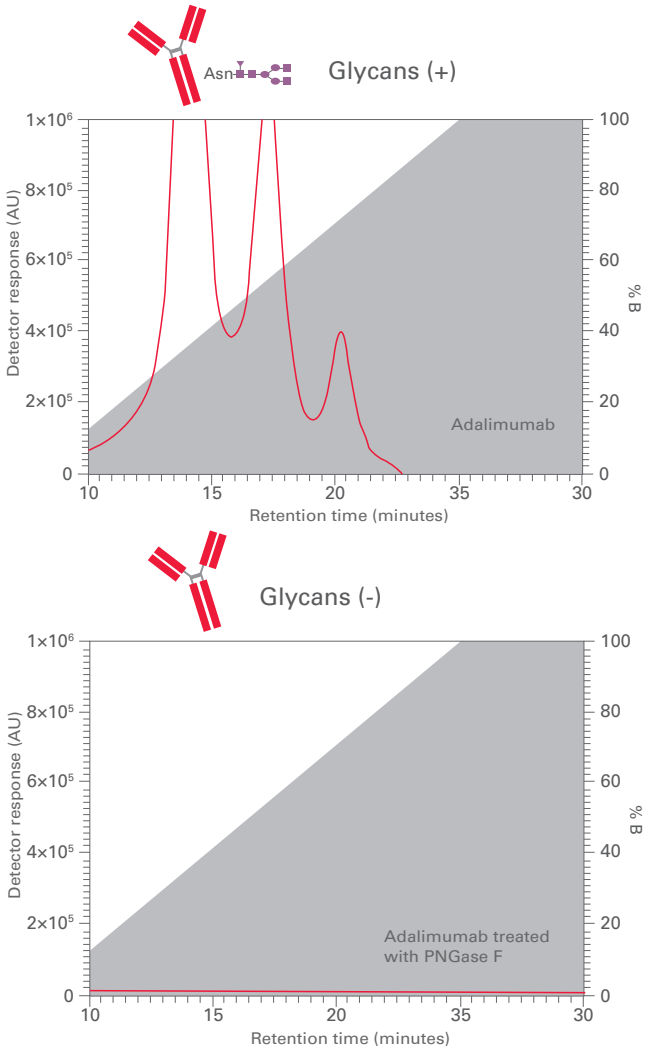
TSKgel FcR-IIIA-NPR is a 4.6 mm ID × 7.5 cm PEEK column for high performance affinity chromatography. Made of PEEK hardware, this column has been designed for the separation of mAb efficacy variants on the basis of affinity of the N-linked glycosylation in the Fc Region of IgG1-Fc for the FcγRIIIa stationary phase. The recombinant, modified, human FcγRIIIa ligand (*E. Coli* expression system, non-glycosylated) is bonded to 5 µm non-porous polymethrylate beads, providing efficient and rapid separation of mAb glycoforms. The rugged nature of the column facilitates analysis both prior to or after purification.

AFFINITY FOR N-GLYCOSYLATED ANTIBODIES

Figure 3 shows the specificity of the recombinant FcγRIIIA ligand for mAbs which contain N-glycans. When adalimumab, a therapeutic antibody against tumor necrosis factor γ, is injected onto the column, three peaks can be resolved, corresponding to the molecule's glycan heterogeneity. De-glycosylated adalimumab obtained by PNGase F treatment, however, is not retained on TSKgel FcR-IIIA-NPR. These results show the affinity of the FcγRIIIA ligand for mAb glycoforms.

FIGURE 3

HPLC ANALYSIS OF ADALIMUMAB WITH AND WITHOUT PNGASE F TREATMENT USING TSKgel FCR-IIIA-NPR



Column:	TSKgel FcR-IIIA-NPR
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	50 µL of adalimumab or PNGase F treated adalimumab (1 g/L)

ANTIBODY AFC
FC RECEPTOR AFFINITY APPLICATIONS

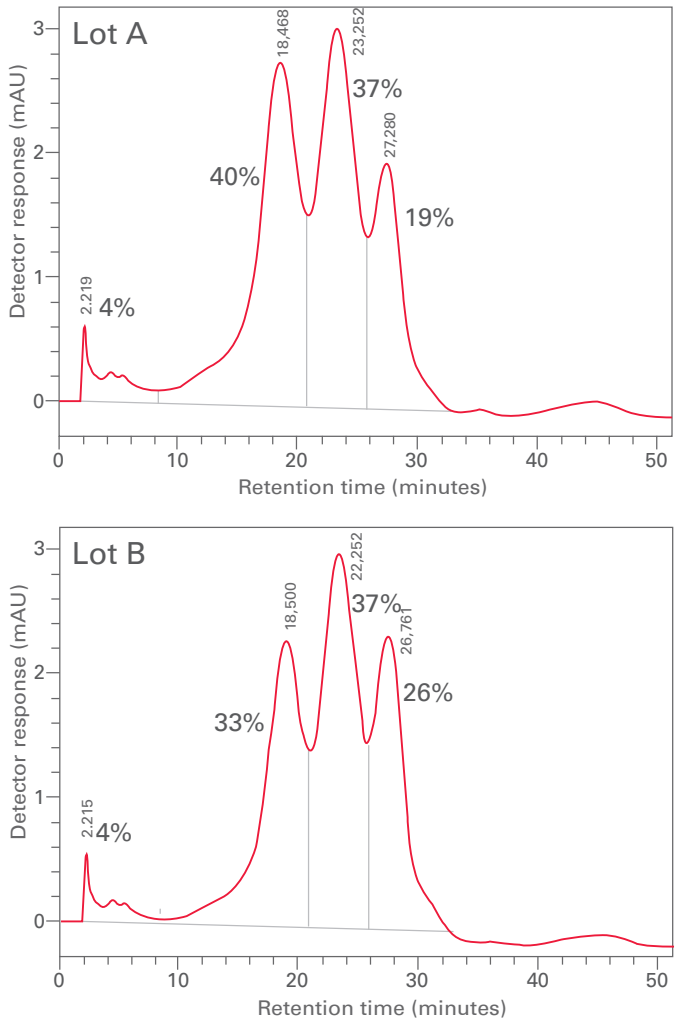
MAb Quality Control

Figure 4 shows the use of the TSKgel FcR-IIIA-NPR column for mAb quality control. Two lots of the same therapeutic monoclonal antibody were injected onto the column for analysis.

Differences in relative peak area percentages indicate that lot-to-lot variations are present. This column can provide a fast and effective way to detect differences in ADCC activity and mAb glycoform prevalence in drug products.

FIGURE 2

TSKgel FCR-IIIA-NPR ELUCIDATES LOT-TO-LOT VARIATION OF A mAb BIOTHERAPEUTIC



Column:	TSKgel FcR-IIIA-NPR
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	mAb based biotherapeutic, Lot A and B

ANTIBODY AFC
FC RECEPTOR AFFINITY APPLICATIONS

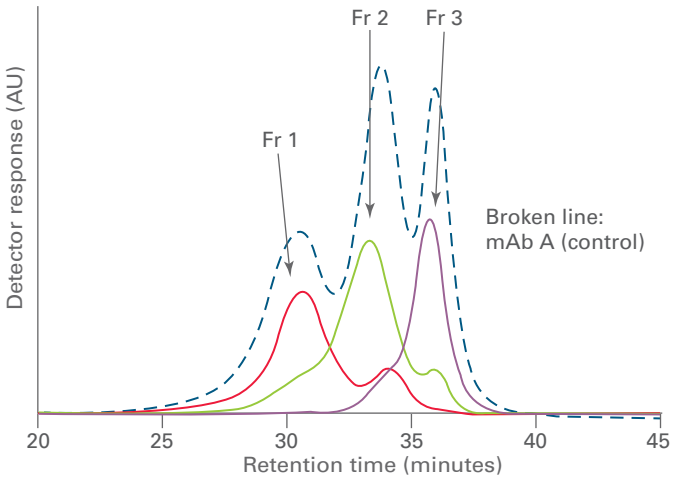
ADCC Efficacy

The affinity of a mAb glycoform for FcγRIIIa is correlated to its ADCC activity. Peak fractions from a typical separation of mAb A were collected and pooled as shown in Figure 5. Figure 6 shows the corresponding ADCC activity of each sample.

As indicated, the most retentive component, fraction 3, displays the highest level of ADCC activity. Each individual fraction shows a different level of ADCC activity than calculated for the entire mAb sample.

FIGURE 2

POOLED FRACTIONS OF mAb A FOR ADCC ANALYSIS

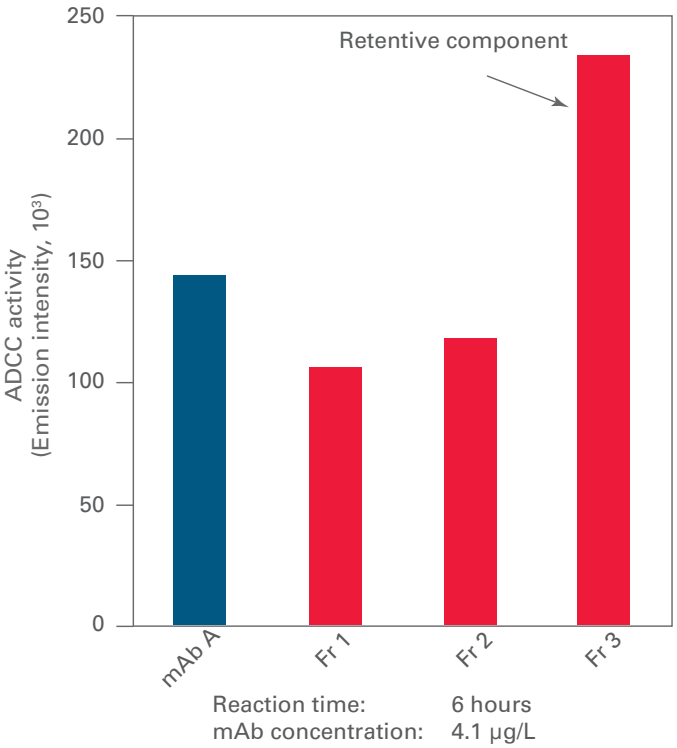


Content (%)

	Peak 1	Peak 2	Peak 3
Control	22	44	34
Fr 1	83	17	0
Fr 2	12	80	8
Fr 3	0	9	91

FIGURE 2

ADCC ACTIVITIES OF EACH FRACTION



Column: TSKgel FcR-IIIA-NPR
Mobile phase A: 50 mmol/L citrate, pH 6.5
Mobile phase B: 50 mmol/L citrate, pH 4.5
Flow rate: 1 mL/min
Detection: UV @ 280 nm
Sample: mAb-based biotherapeutic

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Pore size (nm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel PEEK columns							
0023513	TSKgel FcR-IIIA-NPR	4.6	7.5	5		≥ 170	9.0

ANTIBODY AFC
PROTEIN A AFFINITY CHROMATOGRAPHY

HOW DOES IT WORK?

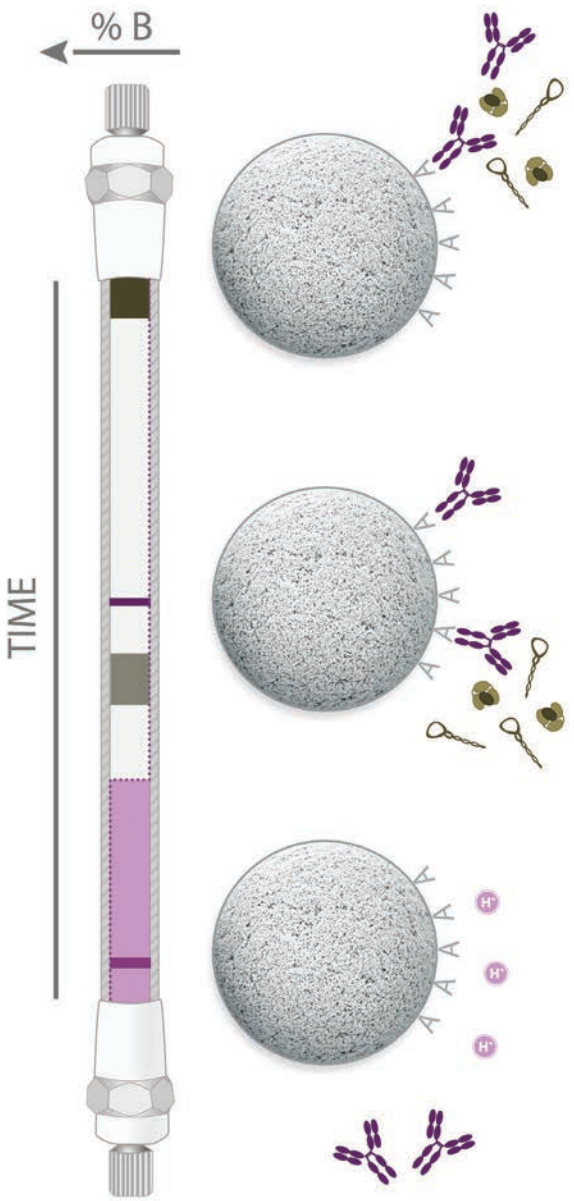
Protein A Chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium Staphylococcus aureus. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region.

While the native form of Protein A was used as the ligand for first generation Protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest.

In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. If necessary, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound IgG is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding.

FIGURE 1

PROTEIN A AFFINITY CHROMATOGRAPHY ILLUSTRATION



ANTIBODY AFC
ABOUT TSKgel Protein A-5PW

TSKgel Protein A-5PW is specifically designed for fast and accurate determination of monoclonal antibody (mAb) concentration

- Wide dynamic range for mAb titer determination
- Fast analysis: 1-2 min/analysis
- Long lifetime: > 2,000 injections per column

TSKgel PROTEIN A-5PW PROPERTIES

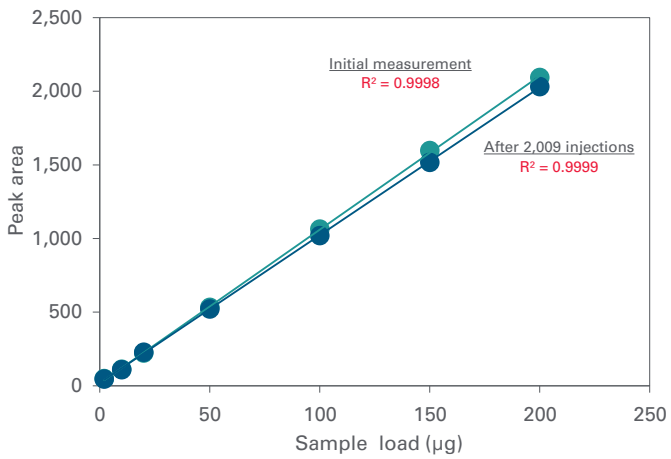
TSKgel Protein A-5PW is a 4.6 mm ID × 3.5 cm column for high performance affinity chromatography. Made of PEEK hardware, this column has been designed for the rapid separation and robust quantification of a variety of antibodies. Monoclonal antibodies can be captured and accurately quantitated in less than two minutes per injection.

The recombinant Protein A ligand, well-known from our TOYOPEARL affinity resins, is a code-modified hexamer of the C domain. This ligand has an affinity for various antibodies that the native protein A and some other recombinant protein A ligands do not possess. For example, it has high affinity for different subclasses of antibodies from rat and goat which native protein A does not have any affinity for.

The recombinant ligand is bound to the 100 nm pore size TSKgel 5PW base bead via multipoint attachment resulting in excellent base stability in 0.1 mol/L NaOH. The resulting low level of Protein A leaching makes this column a good candidate for small scale purification of mAbs for initial characterization in R&D.

FIGURE 1

DURABILITY AND DYNAMIC RANGE OF TSKgel Protein A-5PW



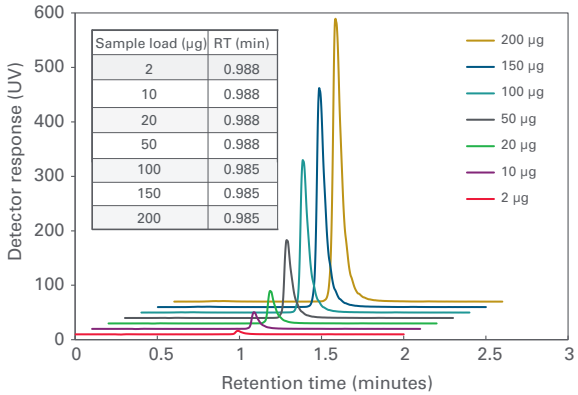
DURABILITY AND WIDE DYNAMIC RANGE

The column can be used for more than 2,000 injections without regeneration or cleaning.

Packed with 20 µm hydroxylated methacrylic polymer beads with a high degree of crosslinking, it allows a high flow rate while still maintaining chromatographic efficiency, peak width and resolution. The high durability and wide dynamic range of TSKgel Protein A-5PW is demonstrated in Figure 1. For linearity analysis different amounts of purified IgG were initially injected onto the column. The column was then used up to 2,009 injections without being cleaned. The linearity analysis was then repeated. No significant change in the calibration curve for IgG was observed. The column still maintained its high loading capacity with an excellent linearity (R2 = 0.9999).

FIGURE 2

WIDE RANGE OF LOADING CONCENTRATIONS OF PURIFIED IgG



Column: TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm L
Binding and washing buffer: 20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer: 20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient: 0 - 0.5 min: binding buffer
0.5 - 1.1 min: elution buffer
1.1 - 2.0 min: binding buffer
Flow rate: 2 mL/min
Detection: UV @ 280 nm
Sample: IgG

ANTIBODY AFC PROTEIN A AFFINITY APPLICATIONS

WIDE DYNAMIC RANGE AND SENSITIVITY OF DETECTION

Determination of mAb concentration from harvested cell culture supernatant requires a column with good linearity over a wide dynamic range. Similar chromatograms from 2 to 200 µg of load without any change of peak profile or retention are produced by this column (Figure 2). The wide range loading capacity of the TSKgel Protein A-5PW column can accurately determine the titer of mAb at various stages of mAb development: from low concentrations during initial screening in R&D to high titers in process control.

ANALYSIS OF mAb TITER

In many stages of mAb development, samples must be screened for IgG titer. TSKgel Protein A-5PW can be employed to determine the concentration of monoclonal antibody for the optimal time for harvest or to identify clones that express the most antibodies. If necessary, a partial purification for further analysis can be accomplished using TSKgel Protein A-5PW.

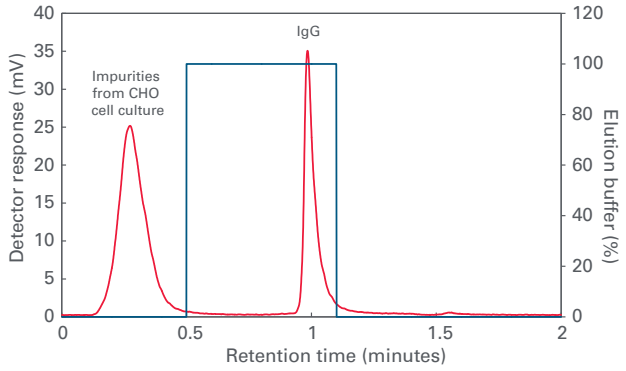
As shown in Figure 3, IgG is separated well from impurities in CHO cell culture supernatant by stepwise pH gradient within two minutes. All host cell proteins from the supernatant are eluted in a flow-through peak and only IgG is captured and eluted by the column.

HIGH FLOW RATE FOR HIGH THROUGHPUT ANALYSIS

Four different flow rates (1, 2, 3 and 4 mL/min) were used to demonstrate the high flow rate performance of the column. Figure 4 shows that the relative peak area percentages of the unbound (flow-through) protein peak and the bound IgG remained unchanged at different flow rates. Less than one minute analysis time was available at 4.0 mL/min.

FIGURE 3

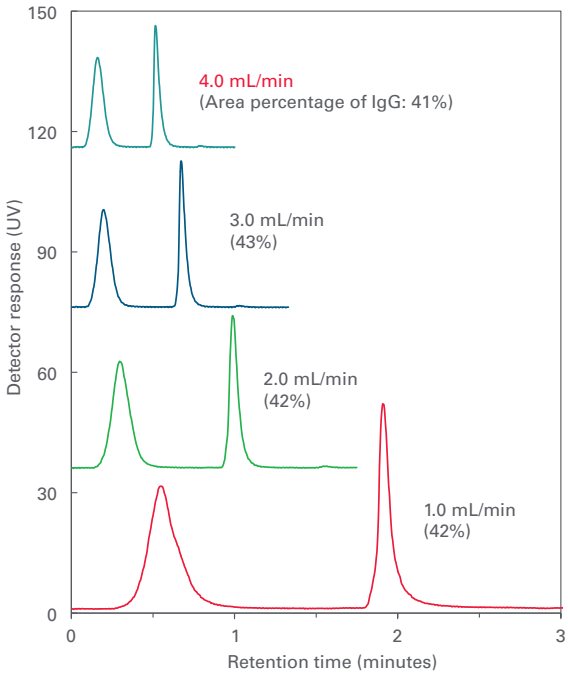
RAPID SEPARATION OF IgG FROM IMPURITIES



Column:	TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm L
Binding buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 – 0.5 min: binding buffer;
	0.5 – 1.1 min: elution buffer;
	1.1 – 2.0 min: binding buffer
Flow rate:	2 mL/min
Detection:	UV @ 280 nm
Sample:	20 µL CHO cell culture supernatant containing polyclonal IgG (0.5 g/L)

FIGURE 4

EFFECT OF FLOW RATE ON SEPARATION



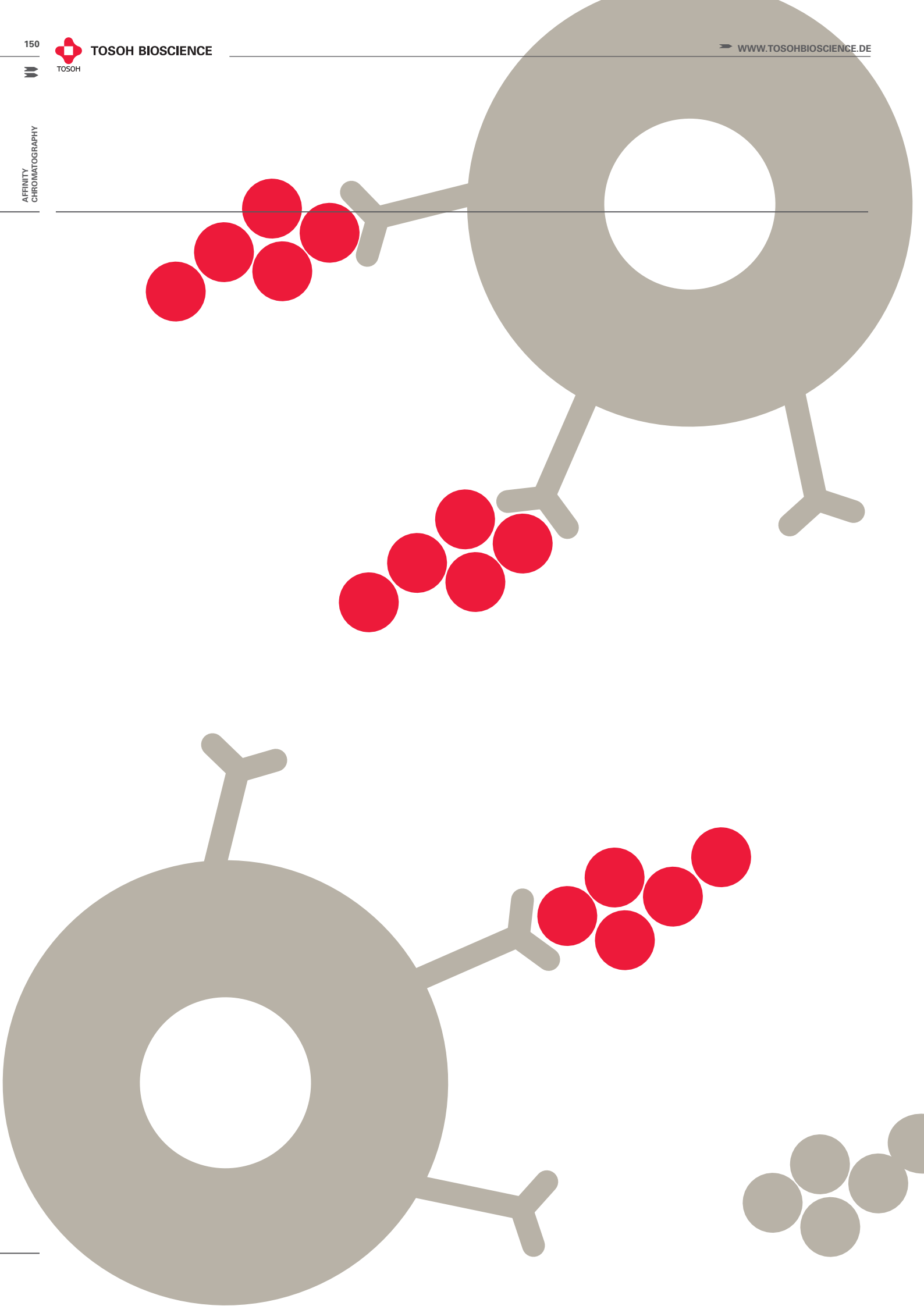
Gradient conditions

Flow rate (mL/min)	Binding buffer (min)	Elution buffer (min)	Binding buffer (min)
4.0	0-0.25	0.25-0.55	0.55-1.00
3.0	0-0.33	0.33-0.73	0.73-1.33
2.0	0-0.50	0.50-1.10	1.10-2.00
1.0	0-1.00	1.00-2.20	2.20-4.00

20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Pore size (nm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel Protein A columns							
0023483	TSKgel Protein A-5PW	4.6	3.5	20	100	≥ 280	2.0



AFC
AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

GROUP SPECIFIC COLUMNS

TSKgel Boronate-5PW
TSKgel Chelate-5PW

ACTIVATED COLUMNS

TSKgel Tresyl-5PW

> TOYOPEARL AF-rProtein L-650F is an innovative and very useful chromatographic resin in my purification toolbox, as it allows capture of multiple antibody types. It is the resin I've been expecting for many years.
>
> Dr. Michael Davids
> Davids Biotechnologie

AFC
HOW DOES IT WORK?

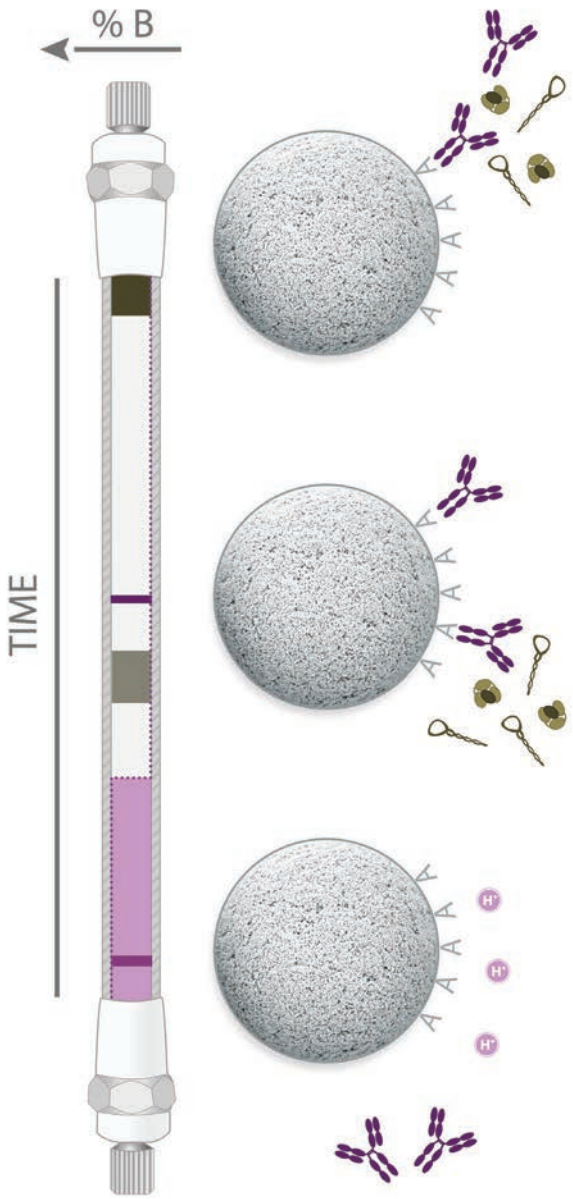
Affinity Chromatography (AFC) offers the greatest potential specificity and selectivity for the isolation or purification of biomolecules. Almost all biological molecules can be purified on the basis of a specific interaction between their chemical or biological structure and a suitable affinity ligand.

In affinity chromatography, the target protein is specifically and reversibly bound by a complementary ligand. The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed out of the column, and bound target protein is eluted by changing conditions to those favoring elution. Elution is performed specifically, using a competitive target, or nonspecifically, by changing, for example, pH, ionic strength, or polarity.

There are many custom designed affinity ligands available to the chromatographer besides antibody affinity columns.

FIGURE 1

AFFINITY CHROMATOGRAPHY ILLUSTRATION



AFC
ABOUT TSKgel AFFINITY COLUMNS

- TSKgel Boronate-5PW binds 1,2 cis-diol groups under alkaline pH conditions
- TSKgel Chelate-5PW loaded with metal ions can bind peptides and proteins containing histidine residues
- TSKgel Tresyl-5PW can be used to create a custom affinity columns by activation with a user-selected ligand containing amino, thiol, phenol, or imidazole groups

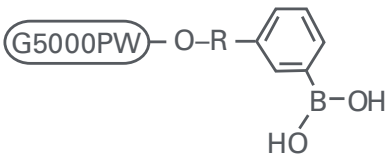
The TSKgel affinity chromatography column line consists of two group-specific stationary phases: Boronate-5PW and Chelate-5PW, as well as one with a chemically-activated functionality, Tresyl-5PW. All analytical TSKgel AFC columns are based on the well-established 10 µm rigid TSKgel G5000PW resin. This resin features 100 nm pores that have an estimated exclusion limit of 1 million Dalton, along with excellent stability from pH 2 to 9.

The structures of the available functional ligands are shown in Figure 2. The choice of a specific ligand is dictated by the expected interaction between the sample and the 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.

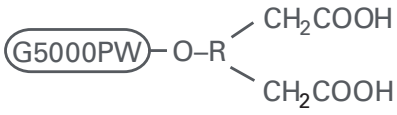
FIGURE 2

TSKgel AFFINITY CHROMATOGRAPHY COLUMN PACKINGS

TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgelTresyl-5PW

FEATURES

- Choice of affinity ligands
- Stable affinity ligands
- Large pore size
- Rigid polymeric base resins

BENEFITS

- Application flexibility, scalability from lab to process
- Robust columns with long lifetime
- Enhanced access of large proteins to affinity ligand
- Wide buffer pH (2-12) range

AFC
ABOUT TSKgel CHELATE-5PW

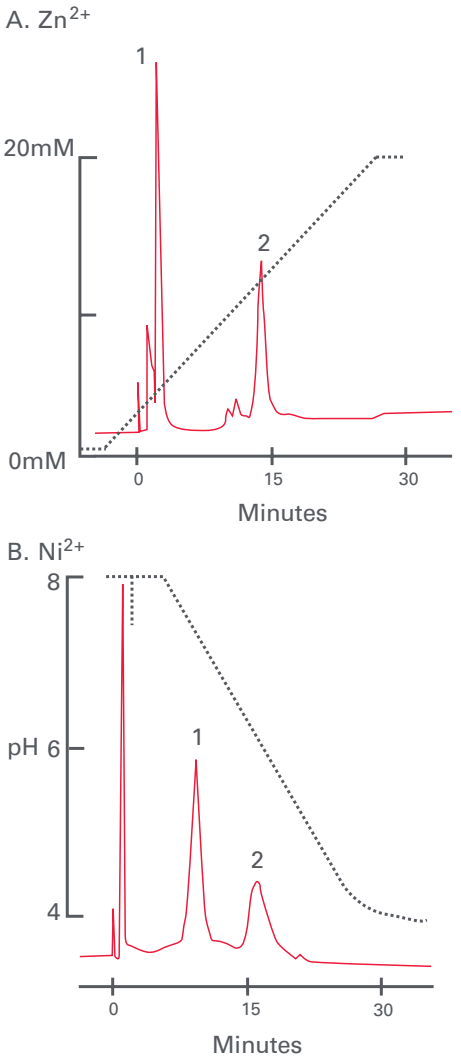
TSKgel Chelate-5PW utilizes the ability of iminodiacetic acid (IDA) to chelate ions such as Zn^{2+}, Ni^{2+} and Cu^{2+}. The column is pre-loaded with divalent metal ions by chelation. Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. The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

Applications for TSKgel Chelate-5PW include the analysis of serum proteins such immunoglobulins and 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.

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+}
Mobile phase:	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
Sample:	1. ribonuclease A (bovine)
	2. transferrin (human)

AFC
ABOUT 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.

TSKgel Boronate-5PW APPLICATIONS

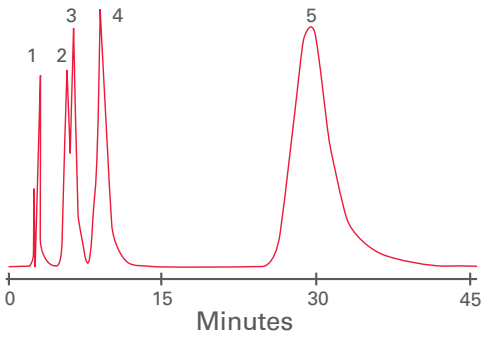
Applications for TSKgel Boronate-5PW include: carbohydrates, nucleic acids, nucleotides, nucleosides, catecholamines, and other biomolecules containing the 1,2 cis-diol functionality.

CATECHOLAMINES

Catecholamines are "fight-or-flight" hormones that are released by the adrenal glands in response to stress. They are called catecholamines because they contain a catechol group and are derived from the amino acid tyrosine. Figure 4 shows the analysis of catecholamines using the TSKgel Boronate-5PW affinity column and phosphate buffer.

FIGURE 4

SEPARATION OF CATECHOLAMINES ON TSKgel Boronate-5PW



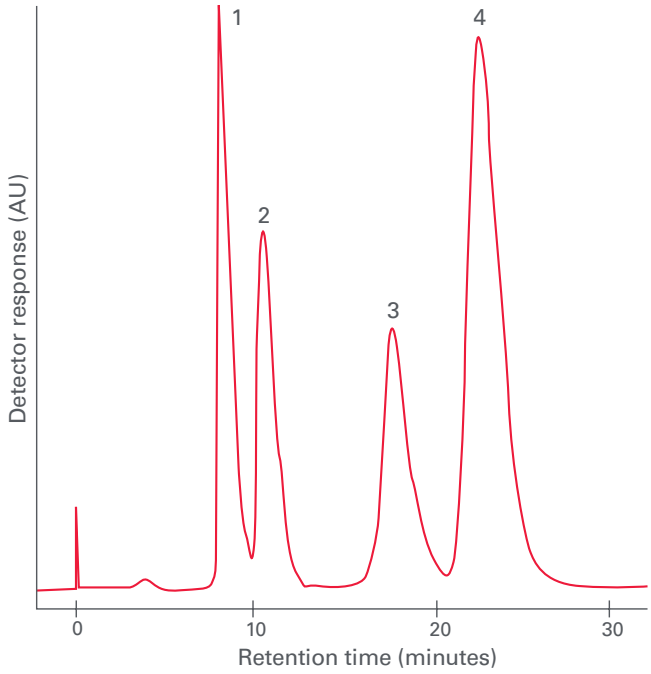
Column:	TSKgel Boronate-5PW, 7.5 mm ID x 7.5 cm L
Mobile phase:	0.1 mol/L phosphate buffer, pH 6.5
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	1. tyrosine
	2. normetanephrine
	3. metanephrine
	4. DOPA
	5. epinephrine

NUCLEOSIDES

Nucleosides are glycosylamines consisting of a nucleobase (often referred to as simply base) bound to a ribose or deoxyribose sugar via a beta-glycosidic linkage. Examples of nucleosides include cytidine, uridine, adenosine, guanosine, thymidine, and inosine. Figure 5 shows the selective separation of nucleosides using a TSKgel Boronate-5PW column and isocratic conditions.

FIGURE 5

ISOCRATIC SEPARATION OF NUCLEOSIDES



Column:	TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm L
Mobile phase:	0.1 mol/L phosphate buffer, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. cytidine
	2. uridine
	3. guanosine
	4. adenosine

AFC
ABOUT TSKgel TRESYL-5PW

Unlike other TSKgel affinity columns, the TSKgel Tresyl-5PW columns, which are derivatized with the 2,2,2-trifluroethanesulfonyl ligand, require 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.

TSKgel Tresyl-5PW APPLICATIONS

Antibody Ligands

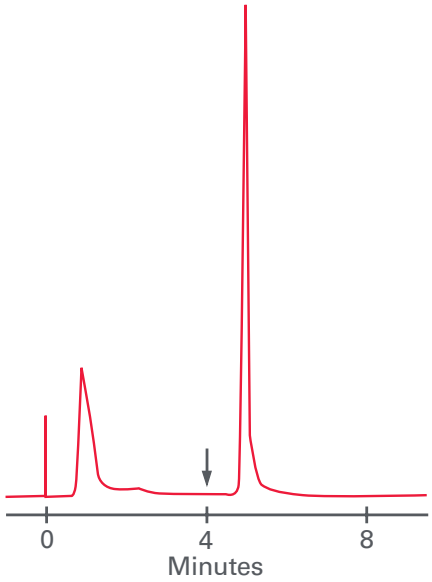
Principal applications for TSKgel Tresyl-5PW columns include the selective purification of antigens after coupling the appropriate antibody to the solid support. The antibody coupling yield at pH >7.5 is more than 90%, with the maximum binding occurring at pH 7.5. Antigen adsorption to the antibody ligand is most effective when the antibody concentration is <2-3 g/L 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 two hours to complete the cross-linking reaction, whereas it may take 6-7 hours to fully attach an antibody at the concentration of 10 g/L resin.

Peroxidase on Concanavalin A

The wide range of applications using TSKgel Tresyl-5PW includes the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins). The chromatogram in Figure 6 shows the purification of peroxidase by the concanvalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

FIGURE 6

PURIFICATION OF PEROXIDASE ON CONCANAVALIN A COUPLED TO TSKgel Tresyl-5PW



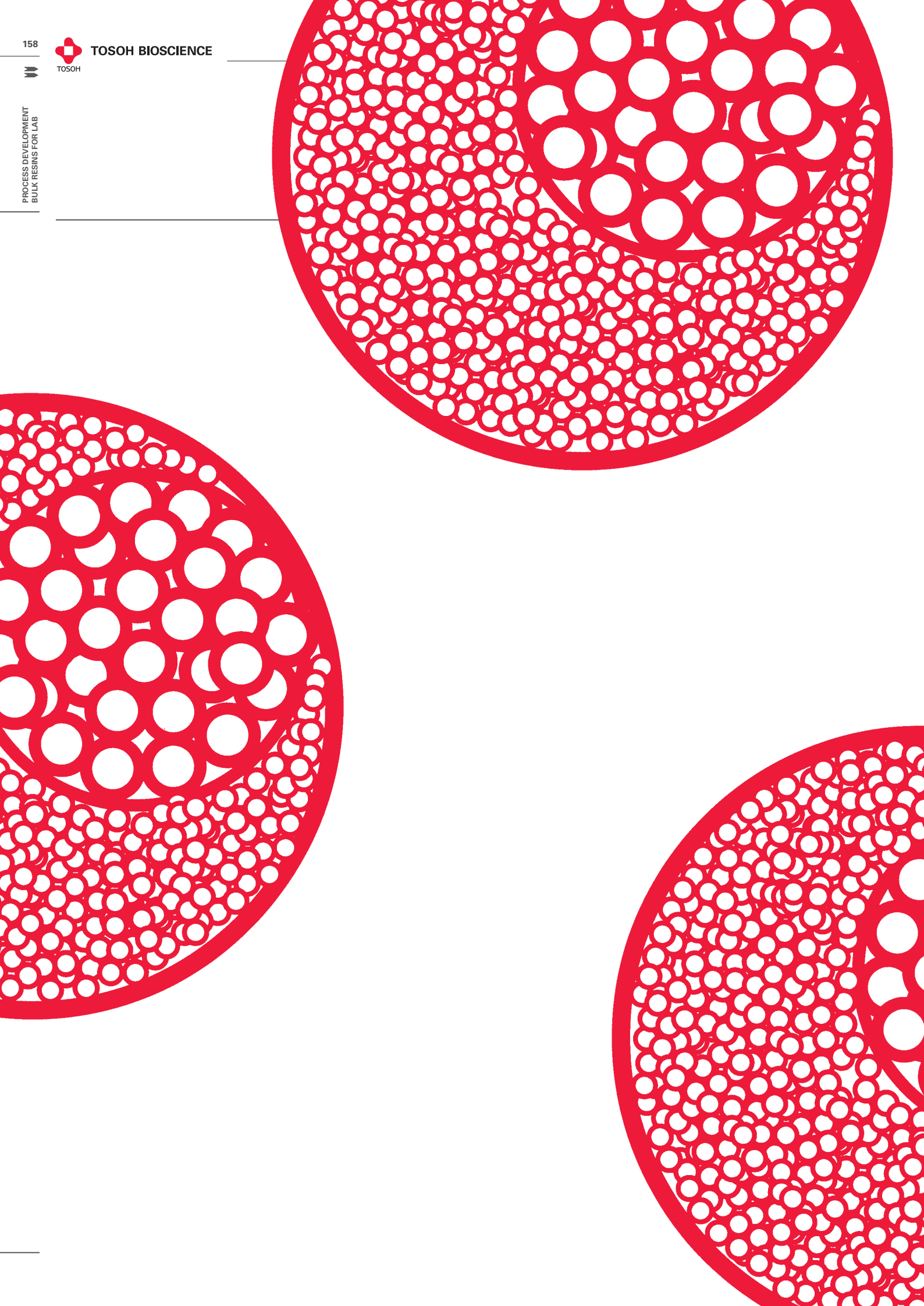
Washing step:	Wash TSKgel Tresyl-5PW, 6 mm ID x 4 cm L, with DI water
Ligand solution:	Dissolve 40 mg of concanavalin A in 10 mL of 0.1 mol/L $NaHCO_3$, pH 8.0, containing 0.5 mol/L NaCl
Coupling step:	Recycle the ligand solution overnight through the column at 0.2 mL/min at 25 °C
Blocking step:	Block 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
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 $MgCl_2$
Mobile phase:	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
Sample:	Crude peroxidase, 0.5 mg

AFC
ORDERING INFORMATION TSKgel AFC COLUMNS

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel Boronate Columns						
0013066	Boronate-5PW	7.5	7.5	10	≥ 1,300	1.0
0014449	Boronate-5PW Glass	5.0	5.0	10	≥ 500	2.0
0013125	Boronate-5PW Guardgel Kit				For P/N 0013066	
0014451	Boronate-5PW Glass Guardgel Kit			20	For P/N 0014449	
TSKgel Chelate Columns						
0008645	Chelate-5PW	7.5	7.5	10	≥ 1,300	1.0
0014440	Chelate-5PW Glass	5.0	5.0	10	≥ 500	2.0
0020022	BioAssist Chelate	7.8	5.0	10	≥ 800	1.0
0008647	Chelate-5PW Guardgel Kit				For P/N 0008645	
TSKgel Tresyl Columns						
0014455	Tresyl-5PW	6.0	4.0	10		1.0
0014456	Tresyl-5PW	7.5	7.5	10		1.0
Tresyl 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

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

For over twenty years our workshops on Chromatography in Process Development and Production provide a comprehensive background to chromatographic purification of biomolecules.

PROCESS DEVELOPMENT HOW DOES IT WORK?

Screening and selection of appropriate chromatography media is an integral part of the development of purification schemes for biomolecules. Due to the diversity in available ligand chemistries and base matrices offered by different vendors (e.g., agarose, methacrylate, styrene/divinylbenzene, etc.), it is prudent at the first part of the development process to screen as many resins as possible.

A thorough evaluation is a necessity as each target molecule has very different physical and chromatographic properties. A resin that worked in the past for a similar molecule might not work as effectively for the new target molecule. In addition, performance parameters such as selectivity, binding capacity, recovery, etc. are mainly influenced by the properties of the chromatographic resin. Therefore, selection of the most suitable resin is the significant key point to succeed in purification.

Tosoh Bioscience offers a wide variety of screening tools composed of TOYOPEARL and TSKgel media. Pre-packed columns such as MiniChrom columns packed with TOYOPEARL or TSKgel can also be used for small scale purifications in R&D. Thea are compatible with every commercial Chromatographysystem. In addition, bulk media volumes of < 1 L that can be packed in appropriate columns dimensions are available for process development and laboratory scale purifications.

TOYOPEARL AND TSKgel PROCESS MEDIA

TOYOPEARL media are hydrophilic porous methacrylic resins for preparative applications. Their rigid polymeric backbone has better pressure-flow properties than most other commercially made materials. Therefore, higher linear operating velocities can be used for faster process throughput and decreased cycling times.

TOYOPEARL resins are stable over the pH 2-12 range for normal operating conditions and pH 1-13 for cleaning conditions. The resins are available in average particle sizes of 35 µm, 65 µm, 75 µm, and 100 µm for high resolution, intermediate purification, or capture chromatography. In most modes, TOYOPEARL is available in three grades: S (superfine) for highest performance, F (fine), and M (medium) for economical purification. Two additional grades, C (coarse) and EC (extra coarse), are available for capture.

TOYOPEARL resins are also offered in many different pore diameters for size exclusion, ion exchange, hydrophobic interaction, mixed-mode, and affinity chromatography. Pore diameter and surface area were optimized to ensure excellent kinetic access and binding capacity of a potential target molecules regardless of molecular size. For predictable results in scale-up, TOYOPEARL resins are based on the same chemistries as the pre-packed TSKgel columns. This allows the seamless direct scale-up of methods developed on TSKgel columns to TOYOPEARL resins.

TSKgel resins are larger particle size versions of the chemically equivalent methacrylic packing of analytical scale TSKgel columns. The resins with particle sizes of 20 µm and 30 µm are available in bulk quantities for large scale ion exchange and hydrophobic interaction chromatography. Their mechanical stability and permeability make them excellent for use when increased separation performance and plate count are needed for optimum preparative or process chromatography.

PROCESS DEVELOPMENT ABOUT MiniChrom COLUMNS

- Easy screening and method optimization
- Broad range of media available
- Suited for laboratory scale purifications
- Fit to any LC-System

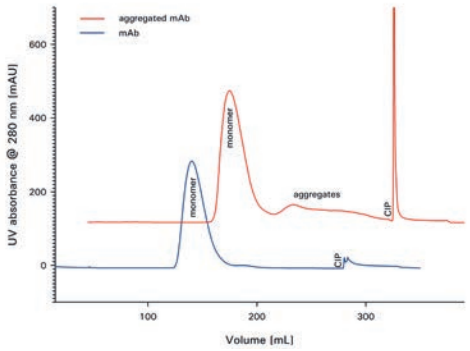
Many TOYOPEARL and TSKgel media are available in the well-known 5 mL MiniChrom format (8 mm ID x 100 mm) for parameter screening, method optimization and/or small scale purifications. The 5 mL MiniChrom columns are the ideal tools to further optimize the purification method and to confirm the operational 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 robotic 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 available with a broad range of ion exchange, hydrophobic interaction, mixed-mode, and Protein A and L affinity resins. See the chapter on bulk resins for detailed information on TOYOPEARL 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 1

MIXED-MODE SEPARATION ON MiniChrom MX-Trp-650M



Column:	MiniChrom MX-Trp-650M, 8 mm ID x 10 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

s

ORDERING INFORMATION

Part #	Description	Package description
Size Exclusion		
0045171	MiniChrom TOYOPEARL HW-40F, 5 mL	8 mm ID x 100 mm L
Ion Exchange		
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

PROCESS DEVELOPMENT
ORDERING INFORMATION MiniChrom COLUMNS

ORDERING INFORMATION

Part #	Description	Package description
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, 5 mL	8 mm ID x 100 mm L
0045110	MiniChrom TOYOPEARL SP-650M, 5 mL	8 mm ID x 100 mm L
0045111	MiniChrom TOYOPEARL SP-650S, 5 mL	8 mm ID x 100 mm L
0045112	MiniChrom TOYOPEARL DEAE-650M, 5 mL	8 mm ID x 100 mm L
0045113	MiniChrom TOYOPEARL DEAE-650S, 5 mL	8 mm ID x 100 mm L
0045114	MiniChrom TOYOPEARL Super Q-650S, 5 mL	8 mm ID x 100 mm L
0045115	MiniChrom TOYOPEARL Q-600C AR, 5 mL	8 mm ID x 100 mm L
0045116	MiniChrom TSKgel SP-5PW (20), 5 mL	8 mm ID x 100 mm L
0045117	MiniChrom TOYOPEARL Sulfate-650F, 5 mL	8 mm ID x 100 mm L
0045181	MiniChrom Toyopearl CM-650M, 5mL	8 mm ID x 100 mm L
0045182	MiniChrom Toyopearl CM-650S, 5 mL	8 mm ID x 100 mm L
0045183	MiniChrom TSKgel SP-3PW (30), 5mL	8 mm ID x 100 mm L
0045184	MiniChrom TSKgel DEAE-5PW (20), 5mL	8 mm ID x 100 mm L
0045185	MiniChrom Toyopearl SP-550C, 5mL	8 mm ID x 100 mm L
0045186	MiniChrom TP MegaCap II SP-550EC, 5ml	8 mm ID x 100 mm L
Hydrophobic Interaction		
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
0045129	MiniChrom TOYOPEARL Hexyl-650C, 5 mL	8 mm ID x 100 mm L
0045130	MiniChrom TSKgel Phenyl-5PW, 5 mL	8 mm ID x 100 mm L
Mixed Mode		
0045151	MiniChrom TOYOPEARL MX-Trp-650M 5 mL	8 mm ID x 100 mm L
0045152	MiniChrom Ca^{++}Pure-HA, 5 mL	8 mm ID x 100 mm L
Affinity		
0045161	MiniChrom TOYOPEARL AF-rProtein A HC-650M 5 mL	8 mm ID x 100 mm L
0045162	MiniChrom TOYOPEARL AF-rProtein L-650M 5 mL	8 mm ID x 100 mm L

PROCESS DEVELOPMENT ABOUT RoboColumns

- Pre-packed columns for use with robotic systems
- High throughput parallel Chromatography
- Automated screenning and evaluation of design space
- Suited for fast microscale purification

Tosoh Bioscience offers TOYOPEARL media now also in the well-known RoboColumn® format packed by Repligen (former Atoll). RoboColumns are miniaturized chromatographic columns pre-packed with the most popular TOYOPEARL ion exchange, mixed-mode, hydrophobic interaction or affinity media. See the chapter on bulk resins for detailed information on TOYOPEARL resins.

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.

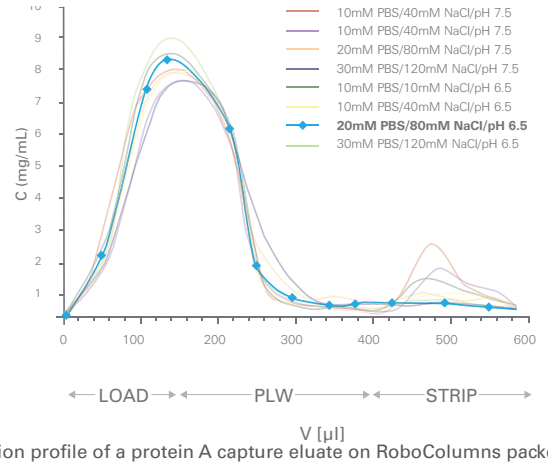
RoboColumns are available in two formats with 200 µL (bed height of 10 mm) and 600 µL (bed height of 30 mm) resin volume, respectively. They are supplied in a row of eight units pre-packed with the same TOYOPEARL resin and sealed with two removable silicon cover seals for proper storage. A 96-well array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

Figure 2 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 2

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, Repligen GmbH.

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		
0045027	RoboColumn Sulfate-650F	0.2 mL*8 cols
0045028	RoboColumn Sulfate-650F	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

PROCESS DEVELOPMENT
ORDERING INFORMATION RoboColumns

ORDERING INFORMATION

Part #	Description	Package description
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
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
Mixed-Mode		
0045051	RoboColumn MX-Trp-650M	0.2 mL*8 cols
0045052	RoboColumn MX-Trp-650M	0.6 mL*8 cols
0045053	RoboColumn Ca^{++}Pure-HA	0.2 mL*8 cols
0045054	RoboColumn Ca^{++}Pure-HA	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
0045089	RoboColumn Butyl-650M	0.2 mL*8 cols
0045090	RoboColumn Butyl-650M	0.6 mL*8 cols
0045091	RoboColumn Hexyl-650C	0.2 mL*8 cols
0045092	RoboColumn Hexyl-650C	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
0045065	RoboColumn AF-rProtein L-650F	0.2 mL*8 cols
0045066	RoboColumn AF-rProtein L-650F	0.6 mL*8 cols

PROCESS DEVELOPMENT ABOUT RESIN SEEKER

- Pre-packed 96-well plate kits
- For use with robotic systems or multi-channel pipettes
- Screenning and evaluation of design space

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL and Ca++Pure-HA resins and are available in several configurations for ion exchange, HIC, mixed-mode, hydroxyapatite, and protein A chromatography. Mixed plates are available for HIC and ion exchange screening (Figure 3).

Resin Seeker 96-well plates can be used to screen multiple steps of the purification process including binding, wash, and elution conditions in addition to resin selectivity, binding kinetics, purity, and recovery of your target molecule.

Resin Seeker 96-well plate kits are manufactured by Orochem and sold by Tosoh Bioscience. All components necessary to run an experiment are included in each kit: a wash plate and collection plate. Resin Seeker plates can be operated manually using a multi-channel pipette or in an automated system designed for high throughput screening in a 96-well plate format.

FIGURE 3

OPTIMIZATION OF ANION EXCHANGE CONDITIONS

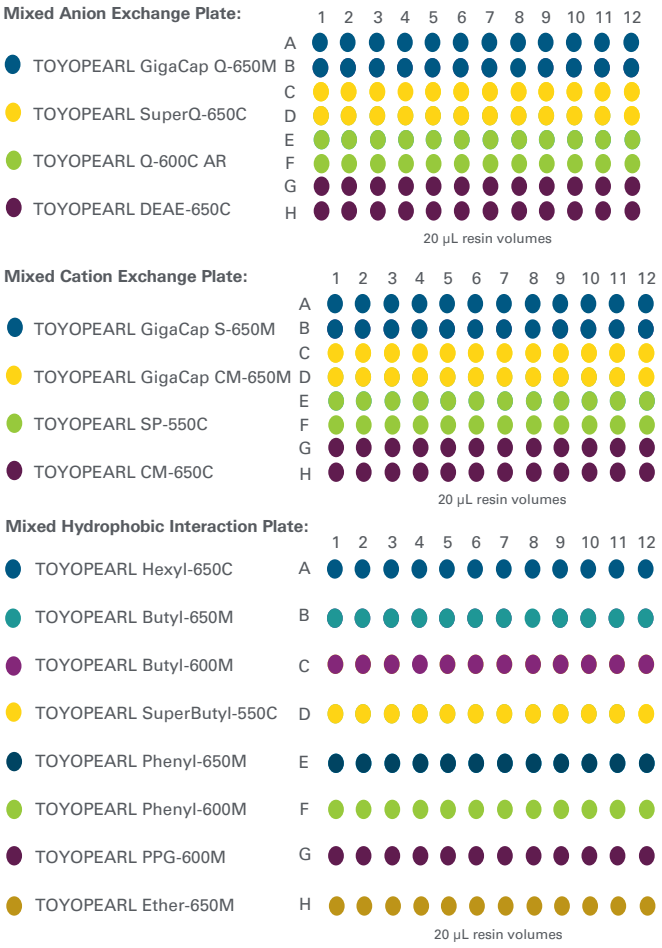


Plate configurations available for Resin Seeker mixed plate offerings

PROCESS DEVELOPMENT
ORDERING INFORMATION RESIN SEEKER

ORDERING INFORMATION

Part #	Description	Matrix	Dimension
Resin Seeker 96 Well Plates for fast automated screening of resins			
0045501	Resin Seeker AIEX	polymer	20 µL 96 well
0045502	Resin Seeker CIEX	polymer	20 µL 96 well
0045503	Resin Seeker GigaCap Q-650M	polymer	20 µL 96 well
0045504	Resin Seeker GigaCap DEAE-650M	polymer	20 µL 96 well
0045005	Resin Seeker GigaCap S-650M	polymer	20 µL 96 well
0045006	Resin Seeker GigaCap CM-650M	polymer	20 µL 96 well
0045007	Resin Seeker NH2-750F	polymer	20 µL 96 well
0045008	Resin Seeker Sulfate-650F	polymer	20 µL 96 well
0045510	Resin Seeker MX-Trp-650M	polymer	20 µL 96 well
0045511	Resin Seeker HIC	polymer	20 µL 96 well
0045509	Resin Seeker AF-rProtein L-650F	polymer	20 µL 96 well
0045520	Resin Seeker AF-rProtein A HC-650F	polymer	20 µL 96 well
0045512	Resin Seeker Ca^{++}Pure-HA	polymer	20 µL 96 well
0045513	Resin Seeker Ca^{++}Pure-HA	polymer	500 µL 96 well

PROCESS DEVELOPMENT ABOUT ToyoScreen

- Pre-packed columns with 1 mL and 5 mL bed volume
- Cartridge design with holder
- Ready to connect with any LC system
- Pack of 5 or 6 pieces in mixed or single chemistry

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.

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.

Initial results from resin screening and optimization with ToyoScreen columns can accurately predict the separation behavior at larger scales.

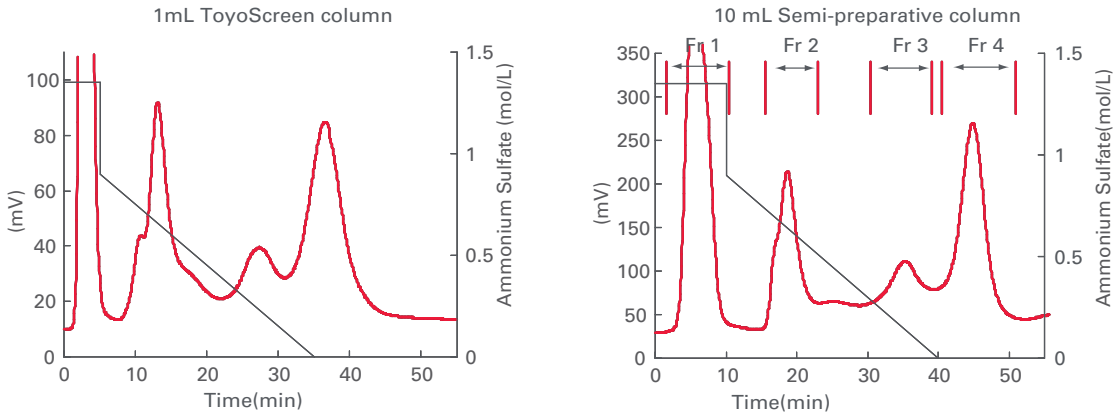
Figure 4 illustrates a practical antibody scale up in which conditions were set using a 1 mL ToyoScreen column and applied to a 10 mL semi-preparative column with a different inner diameter and length.

Similar resolution results are predicted by the following equation:

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

FIGURE 4

COMPARISON CHROMATOGRAMS BETWEEN ToyoScreen AND SEMI-PREPARATIVE COLUMNS



Packing: TOYOPEARL Phenyl-650M; Mobile phase: (A) 0.1 mol/L phosphate buffer containing 1.8 mol/L (NH4)2SO4, 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.*

PROCESS DEVELOPMENT
ToyoScreen APPLICATIONS

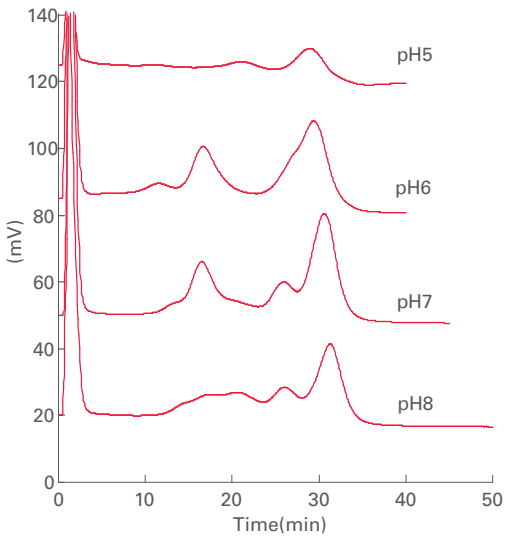
APPLICATIONS

Screening Method Conditions for HIC

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 5 & 6 for anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

FIGURE 5

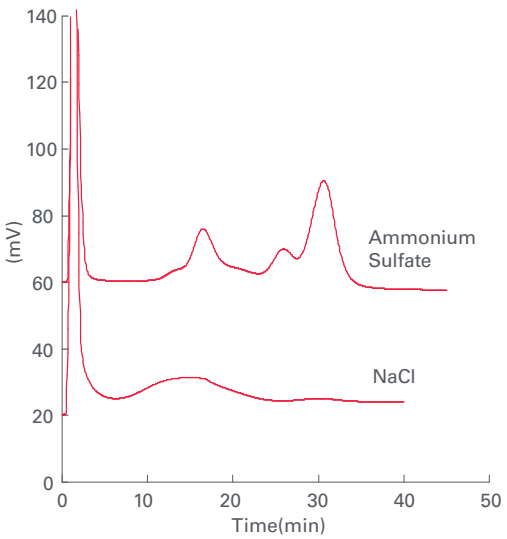
OPTIMIZING ELUENT PH IN HIC



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

FIGURE 6

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)
Injection vol.:	200 µL
Sample:	Cell culture supernatant (x 4 diluted) (antibody: Anti-TSH)

PROCESS DEVELOPMENT
ORDERING INFORMATION ToyoScreen

ORDERING INFORMATION

Part #	Description	Package description
Cation Exchange		
0023472	ToyoScreen Sulfate-650F	1 mL x 6 ea
0023473	ToyoScreen Sulfate-650F	5 mL x 6 ea
0021868	ToyoScreen GigaCap S-650M	1 mL x 6 ea
0021869	ToyoScreen GigaCap S-650M	5 mL x 6 ea
0021368	ToyoScreen SP-650M	1 mL x 6 ea
0021369	ToyoScreen SP-650M	5 mL x 6 ea
0021370	ToyoScreen SP-550C	1 mL x 6 ea
0021371	ToyoScreen SP-550C	5 mL x 6 ea
0021870	ToyoScreen MegaCap II SP-550EC	1 mL x 6 ea
0021871	ToyoScreen MegaCap II SP-550EC	5 mL x 6 ea
0021951	ToyoScreen GigaCap CM-650M	1 mL x 6 ea
0021952	ToyoScreen GigaCap CM-650M	5 mL x 6 ea
0021366	ToyoScreen CM-650M	1 mL x 6 ea
0021367	ToyoScreen CM-650M	5 mL x 6 ea
0021396	ToyoScreen IEC Mix Pack (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	1 mL x 6 Grades x 1 ea
0021397	ToyoScreen IEC Mix Pack (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	5 mL x 6 Grades x 1 ea
0021394	ToyoScreen IEC Cation Mix Pack (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	1 mL x 5 Grades
0021395	ToyoScreen IEC Cation Mix Pack (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	5 mL x 5 Grades
Anion Exchange		
0023443	ToyoScreen NH_2-750F	1 mL x 6 ea
0023444	ToyoScreen NH_2-750F	5 mL x 6 ea
0022873	ToyoScreen GigaCap DEAE-650M	1 mL x 6 ea
0022872	ToyoScreen GigaCap DEAE-650M	5 mL x 6 ea
0021859	ToyoScreen GigaCap Q-650M	1 mL x 6 ea
0021860	ToyoScreen GigaCap Q-650M	5 mL x 6 ea
0021992	ToyoScreen Q-600C AR	1 mL x 6 ea
0021993	ToyoScreen Q-600C AR	5 mL x 6 ea
0021360	ToyoScreen DEAE-650M	1 mL x 6 ea
0021361	ToyoScreen DEAE-650M	5 mL x 6 ea
0021362	ToyoScreen SuperQ-650M	1 mL x 6 ea
0021363	ToyoScreen SuperQ-650M	5 mL x 6 ea
0021364	ToyoScreen QAE-550C	1 mL x 6 ea
0021365	ToyoScreen QAE-550C	5 mL x 6 ea
0021392	ToyoScreen IEC Anion Mix Pack (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	1 mL x 5 Grades
0021393	ToyoScreen IEC Anion Mix Pack (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	5 mL x 5 Grades
Mixed-Mode		
0022824	ToyoScreen MX-Trp-650M	1 mL x 6 ea
0022825	ToyoScreen MX-Trp-650M	5 mL x 6 ea

PROCESS DEVELOPMENT
ORDERING INFORMATION ToyoScreen

ORDERING INFORMATION

Hydrophobic Interaction		
0021380	ToyoScreen PPG-600M	1 mL x 6 ea
0021381	ToyoScreen PPG-600M	5 mL x 6 ea
0021892	ToyoScreen Phenyl-600M	1 mL x 6 ea
0021893	ToyoScreen Phenyl-600M	5 mL x 6 ea
0021494	ToyoScreen Butyl-600M	1 mL x 6 ea
0021495	ToyoScreen Butyl-600M	5 mL x 6 ea
0021382	ToyoScreen SuperButyl-550C	1 mL x 6 ea
0021383	ToyoScreen SuperButyl-550C	5 mL x 6 ea
0021372	ToyoScreen Ether-650M	1 mL x 6 ea
0021373	ToyoScreen Ether-650M	5 mL x 6 ea
0021374	ToyoScreen Phenyl-650M	1 mL x 6 ea
0021375	ToyoScreen Phenyl-650M	5 mL x 6 ea
0021376	ToyoScreen Butyl-650M	1 mL x 6 ea
0021377	ToyoScreen Butyl-650M	5 mL x 6 ea
0021378	ToyoScreen Hexyl-650C	1 mL x 6 ea
0021379	ToyoScreen Hexyl-650C	5 mL x 6 ea
0021398	ToyoScreen HIC Mix Pack (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	1 mL x 6 Grades x 1 ea
0021399	ToyoScreen HIC Mix Pack (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	5 mL x 6 Grades x 1 ea

Affinity		
0023494	ToyoScreen AF-rProtein L-650F	1 mL x 5 ea
0023495	ToyoScreen AF-rProtein L-650F	5 mL x 1 ea
0023496	ToyoScreen AF-rProtein L-650F	5 mL x 5 ea
0023430	ToyoScreen AF-rProtein A HC-650F	1 mL x 5 ea
0023431	ToyoScreen AF-rProtein A HC-650F	5 mL x 1 ea
0023432	ToyoScreen AF-rProtein A HC-650F	5 mL x 5 ea
0022809	ToyoScreen AF-rProtein A-650F	1 mL x 5 ea
0022810	ToyoScreen AF-rProtein A-650F	5 mL x 1 ea
0022811	ToyoScreen AF-rProtein A-650F	5 mL x 5 ea
0021384	ToyoScreen AF-Chelate-650M	1 mL x 6 ea
0021385	ToyoScreen AF-Chelate-650M	5 mL x 6 ea
0021390	ToyoScreen AF-Heparin HC-650M	1 mL x 6 ea
0021391	ToyoScreen AF-Heparin HC-650M	5 mL x 6 ea
0021388	ToyoScreen AF-Red-650M	1 mL x 6 ea
0021389	ToyoScreen AF-Red-650M	5 mL x 6 ea

ToyoScreen accessories		
0021400	ToyoScreen column holder	

PROCESS DEVELOPMENT ABOUT LABPAK MEDIA

- Selection of media for a particular mode
- Economical small volume packs
- For individual small scale experiments

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.

The resin amounts in LabPak products allow the packing of wider bore and longer columns than available in the ToyoScreen products.

ORDERING INFORMATION

Part #	Description	Container size
TSKgel Labpaks		
on Exchange		
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

PROCESS DEVELOPMENT ABOUT TOYOPEARL/TSKgel BULK MEDIA

- Selection of media for a particular mode
- Economical small volume packs
- For individual small scale experiments

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 RESINS

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 has a high mechanically stability, which simplifies column packing by reducing the setup time and improving reproducibility from column to column. The media are 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.

TOYOPEARL HW-type resins, available in pore sizes ranging from 5 nm to >100 nm, are employed in size exclusion chromatography (SEC). Some TOYOPEARL HW resins are used as starting materials for the production of all other functionalized TOYOPEARL resins.

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

TSKgel 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 affinity 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.

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.

PROCESS DEVELOPMENT
ABOUT TOYOPEARL/TSKgel BULK MEDIA

Mode	Resin	Grade/particle size (µm)	Pore size (nm)**	MW range Proteins (Da)	Operating pH range
SEC	TOYOPEARL HW-40	S (20-40), F (30-60), C(50-100)	5	1×10^2 - 1×10^4	2–12
	TOYOPEARL HW-50	S (20-40), F (30-60)	12.5	5×10^2 - 8×10^4	2–12
	TOYOPEARL HW-55	S (20-40), F (30-60)	50	1×10^3 - 7×10^5	2–12
	TOYOPEARL HW-65	S (20-40), F (30-60)	100	4×10^4 - 5×10^6	2–12
	TOYOPEARL HW-75	S (20-40), F (30-60)	> 100	5×10^5 - 5×10^7	2–12
IEC	TSKgel SuperQ-5PW	20 and 30	100	< 5×10^6	2–12
	TSKgel DEAE-5PW	20 and 30	100	< 5×10^6	2–12
	TSKgel SP-5PW	20 and 30	100	< 5×10^6	2–12
	TSKgel SP-3PW	30	25	< 1×10^4	2–12
	TOYOPEARL Sulfate-650F	F (30-60)	100		
	TOYOPEARL SuperQ-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL DEAE-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL GigaCap Q-650	S (20-50), M (50-100)	100	< 5×10^6	2–12
	TOYOPEARL GigaCap DEAE-650	M (50-100)	100	< 5×10^6	2–12
	TOYOPEARL SP-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL CM-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL GigaCap S-650	S (20-50), M (50-100)	100	< 5×10^6	2–12
	TOYOPEARL GigaCap CM-650	M (50-100)	100	< 5×10^6	2–12
	TOYOPEARL QAE-550	C (50-150)	50	< 5×10^5	2–12
	TOYOPEARL Q-600C AR	C (50-150)	75	< 2.5×10^6	2-12
	TOYOPEARL NH2-750	F (30-60)	>1000	< 5×10^7	2–12
	TOYOPEARL SP-550	C (50-150)	50	< 5×10^5	2–12
MMC	TOYOPEARL MX-Trp-650M	M (50-100)	100	< 5×10^6	2–12
HIC	TSKgel Ether-5PW	20 and 30	100	< 5×10^6	2–12
	TSKgel Phenyl-5PW	20 and 30	100	< 5×10^6	2–12
	TOYOPEARL Ether-650	S (20-50), M (40-90)	100	< 5×10^6	2–12
	TOYOPEARL PPG-600	M (40-90)	75	< 5×10^6	2–12
	TOYOPEARL Phenyl-600	M (40-90)	75	< 5×10^6	2–12
	TOYOPEARL Butyl-600	M (40-90)	75	< 5×10^6	2–12
	TOYOPEARL Phenyl-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL Butyl-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12
	TOYOPEARL Super Butyl-550	C (50-150)	50	< 5×10^5	2–12
	TOYOPEARL Hexyl-650	C (50-150)	100	< 5×10^6	2–12
AFC	TSKgel Tresyl-5PW	10	100	< 5×10^6	2–12
	TOYOPEARL AF-rProtein L-650F	F (30-60)	100	< 5×10^6	N/A
	TOYOPEARL AF-Chelate-650	M (40-90)	100	< 5×10^6	2–12
	TOYOPEARL AF-rProtein A HC-650	F (30-60)	100	< 5×10^6	N/A
	TOYOPEARL AF-Tresyl-650	M (40-90)	100	< 5×10^6	N/A
	TOYOPEARL AF-Epoxy-650	M (40-90)	100	< 5×10^6	N/A
	TOYOPEARL AF-Formyl-650	M (40-90)	100	< 5×10^6	6-9
	TOYOPEARL AF-Amino-650	M (40-90)	100	< 5×10^6	2-12
	TOYOPEARL AF-Carboxy-650	M (40-90)	100	< 5×10^6	2-12
	TOYOPEARL AF-Red-650	M (40-90)	100	< 5×10^6	4-9
	TOYOPEARL AF-Heparin HC-650	M (40-90)	100	< 5×10^6	5-10

*** nominal values; Pore size of base matrix*

PROCESS DEVELOPMENT ABOUT SEC BULK MEDIA

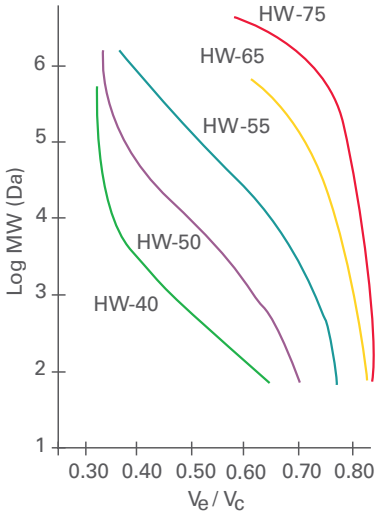
- Pore sizes ranging from 5 nm to >100 nm
- Three particle sizes (S, F, C)
- HW-40 is ideal for desalting applications
- Easy to pack in semi-preparative and process scale columns

Size Exclusion Chromatography (SEC) is a common technique for separating molecules based on their apparent molecular size (their hydrodynamic volume). For over 30 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 I provides an overview of the TOYOPEARL SEC resin product line including corresponding molecular weight ranges of common target samples. Ordering information for quantities < 1 L is provided at the end of this section. Calibration curves of the TOYOPEARL HW-type resins determined with globular proteins are presented in Figure 7.

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 7

CALIBRATION CURVES FOR GLOBULAR PROTEINS



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

TABLE I

PROPERTIES AND MOLECULAR WEIGHT SEPARATION RANGES FOR TOYOPEARL HW-TYPE RESINS

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

(HW = Hydrophilic, water-compatible polymeric base resins)

PROCESS DEVELOPMENT ABOUT ION EXCHANGE BULK MEDIA

- TOYOPEARL GigaCap high capacity ion exchange resins
- TSKgel Super Q -5PW for oligonucleotide purification
- Salt tolerant Anion and Cation Exchanger
- Weak and strong ion exchange ligands available

Ion Exchange Chromatography (IEC) is known for its high resolution and high capacity when it comes to separating mixtures of biomolecules. 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.

EC is often used as a purification step before HIC, SEC, and RPC. IEC is able to 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.

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 II for a complete listing of available particle sizes. Ordering information for quantities < 1 L is provided at the end of this section.

TABLE II

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 NH_2-750F	ST	45
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
TOYOPEARL 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 Sulfate-650F	ST	45
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; ST = Salt tolerant*

PROCESS DEVELOPMENT
ION EXCHANGE PREPARATIVE APPLICATIONS

APPLICATIONS

Scale up of a Anion Exchange purification step

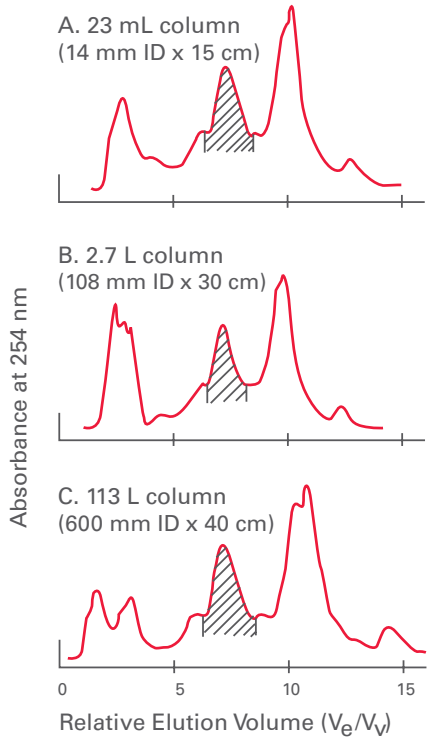
A 5000-fold scale-up of a α-galactosidase enzyme purification was accomplished using TOYOPEARL DEAE-650M. The chromatograms in Figure 8 demonstrate the excellent scale up characteristics of TOYOPEARL ion exchange media. Gradient slope and particle diameter remained unchanged. Linear velocity was reduced 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 to 60 cm). This example illustrates how TOYOPEARL media can be conveniently scaled up from laboratory to production scale applications using the same particle size if desired.

Purification of Oligonucleotides with TSKgel Resins

Resins with SuperQ functionalities are ideally suited for oligonucleotide purification. TSKgel SuperQ-5PW products typically have 2-4 times the binding capacity of other small particle anion exchange resins available on the market. Figure 9 shows the separation of a crude phosphorothioate deoxyoligonucleotide. The N-1 peak can be resolved with TSKgel SuperQ-5PW (20).

FIGURE 8

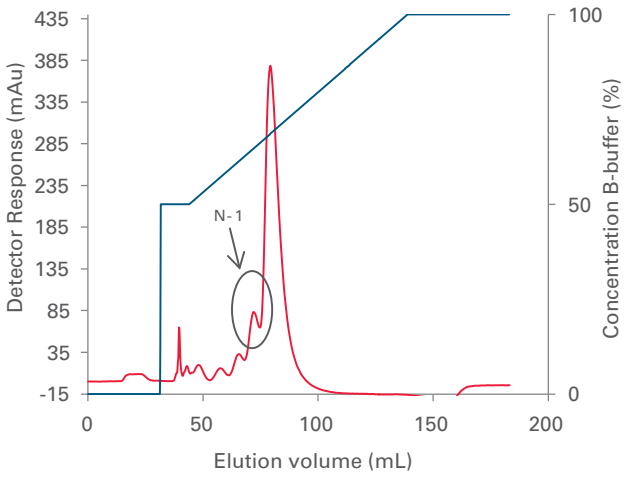
PROCESS SCALE-UP PURIFICATION OF β-GALACTOSIDASE



Column:	TOYOPEARL DEAE-650 M
Mobile phase:	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 @ 254 nm
Sample:	1 % β-galactosidase: A. 8 mL; B. 1 L; C. 40 L

FIGURE 9

PURIFICATION OF OLIGONUCLEOTIDES



Resin:	TSKgel SuperQ-5PW (20)
Column size:	6.6 mm ID × 18.5 cm L (6.3 mL)
Mobile phase:	A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	50 % B (2 CV) 50-100 % B (15 CV), 100 % B (2 CV)
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 254 nm
Sample load:	1.0 mg
Sample:	crude phosphorothioate deoxyoligonucleotide

PROCESS DEVELOPMENT ABOUT MIXED-MODE BULK MEDIA

- Multimodal TOYOPEARL MX-Trp cation exchange resin
- High binding capacity for IgG and other proteins
- Tolerates high conductivity feedstocks
- Sharp elution peaks with mild conditions

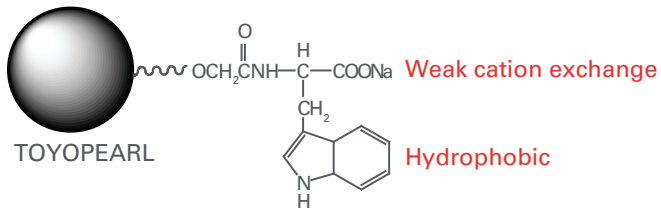
Multimodal or Mixed-Mode Chromatography expands the range of chromatographic modes applied in biopurification. Mixed-mode media combine ionic and hydrophobic interactions and offer new selectivities and a higher salt tolerance than traditional ion exchange media. Mixed-mode media can be used for direct processing of clarified feedstocks at physiological salt concentrations as well as for intermediate and polishing applications. The salt tolerance of the recently introduced TOYOPEARL NH2-750F 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 (Figure 10) is a multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities (Figure 11) 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.

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

FIGURE 10

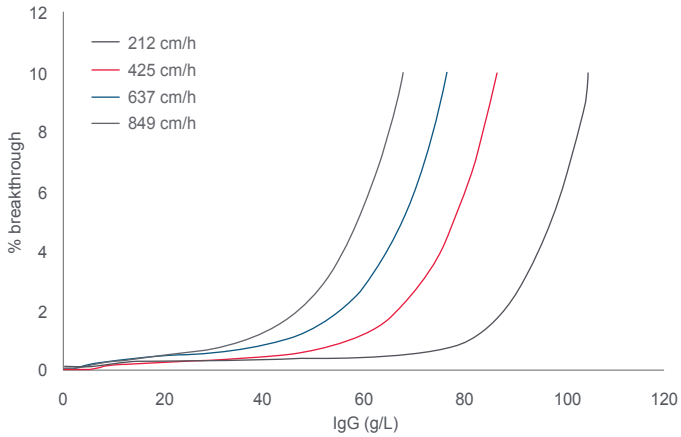
TOYOPEARL MX-Trp-650M STRUCTURE



Product name: TOYOPEARL MX-Trp-650M
Particle size: 50-100 µm

FIGURE 11

IgG BINDING CAPACITY



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

PROCESS DEVELOPMENT ABOUT HIC BULK MEDIA

- Wide range of hydrophobicities, suitable for most proteins
- Standard 100 nm pore size for large biopolymers
- TOYOPEARL "600M" series with optimized pore size for antibody separation
- 3 Butyl pore sizes (50 nm, 75 nm and 100 nm) available
- Seamless scale up from TSKgel 5PW-type to TOYOPEARL

Hydrophobic Interaction Chromatography (HIC) has become a popular mode of chromatography for the purification of biopolymers at analytical as well as preparative scale. HIC is accomplished by the interaction of hydrophobic ligands with the hydrophobic patches 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 media are offered as LABPAK kits in quantities < 1 L and in a combination of resins with varying functionalities.

APPLICATIONS

HIC resins can be applied to separate/purifiy proteins with similar chemical or structural properties, plasmids and monoclonal antibodies. See Figure 12 for separation of large glycoprotein from crude extract on TOYOPEARL Butyl-650S. Please visit our website: www.tosohbioscience.de for extensive application data.

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

TABLE IV

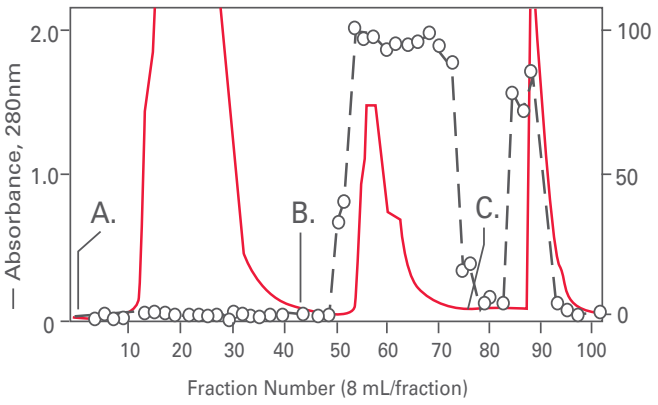
TOYOPEARL AND TSKgel HIC RESINS

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*

FIGURE 12

LARGE GLYCOPROTEIN PURIFIED ON TOYOPEARL BUTYL-650S



Column: TOYOPEARL Butyl-650S, 22 mm ID x 26 cm L
Mobile phase: 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 $(NH_4)_2SO_4$
Sample: crude protein from sea hare Aplysia kurodai

PROCESS DEVELOPMENT ABOUT AFC BULK MEDIA

- High capacity AF-rProtein A-HC resin for antibody purification
- High capacity AF-rProtein L resin for purification of mAb fragments
- Active, reactive and group specific resins
- Provided in standard 100 nm pore size for high capacity of large biopolymers

TOYOPEARL media for Affinity Chromatography (AFC) are based on TOYOPEARL HW-65 resin and functionalized with either group-specific ligands or chemically active groups. Group specific ligands such as Protein A or Protein L specifically bind a selected group of targets such as antibodies and result in a very high purity. 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. The structures of TOYOPEARL activated and reactive ligands are given in Figure 13.

TOYOPEARL AF-rProtein A HC-650F is 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 14). 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.

TOYOPEARL AF-rProtein L-650F is an AFC resin that combines a rigid polymer matrix with a recombinant ligand, which is derived from the B4 domain of native Protein L from peptostreptococcus magnus and is expressed in *E.coli* (Figure 15). Code optimization of the domain results in higher binding capacity and improved stability of the ligand compared to the native molecule.

FIGURE 13

ACTIVATED AND REACTIVE TOYOPEARL AFFINITY RESINS

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(O)-CH_2
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

FIGURE 14

RECOMBINANT PROTEIN A DERIVED LIGAND

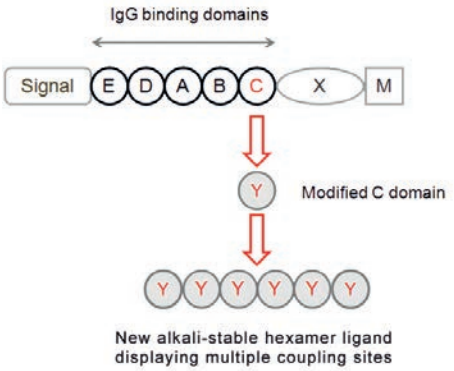
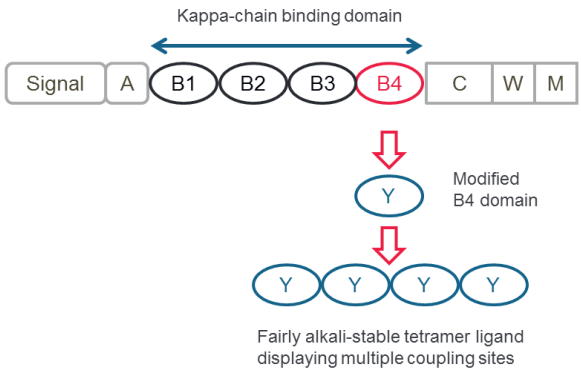


FIGURE 15

KAPPA CHAIN BINDING DOMAINS



PROCESS DEVELOPMENT ORDERING INFORMATION BULK MEDIA

ORDERING INFORMATION

Part #	Description	Container size
A. Size Exclusion Chromatography		
TOYOPEARL bulk resins		
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, 30 µm	150 mL
0007455	HW-50S, 30 µm	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	150 mL
0007466	HW-65C, 75 µm	500 mL
0019816	HW-75F, 45 µm	150 mL
0007469	HW-75F, 45 µm	500 mL
B. Anion Exchange Chromatography		
TSKgel bulk resins		
0043383	SuperQ-5PW (20)	25 mL
0018535	SuperQ-5PW (20)	250 mL
0043283	SuperQ-5PW (30)	25 mL
0018536	SuperQ-5PW (30)	250 mL
0043381	DEAE-5PW (20)	25 mL
0014710	DEAE-5PW (20)	250 mL
0043281	DEAE-5PW (30)	25 mL
0014712	DEAE-5PW (30)	250 mL
TOYOPEARL bulk resins		
0023438	NH_2-750F, 45 µm	100 mL
0023439	NH_2-750F, 45 µm	250 mL
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
C. Cation Exchange Chromatography		
TSKgel bulk resins		
0021976	SP-3PW (30)	25 mL
0021977	SP-3PW (30)	250 mL
0043382	SP-5PW (20)	25 mL
0014714	SP-5PW (20)	250 mL
0043282	SP-5PW (30)	25 mL
0014716	SP-5PW (30)	250 mL
TOYOPEARL bulk resins		
0023467	Sulfate-650F, 100 µm	100 mL
0023468	Sulfate-650F, 100 µm	250 mL
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
0021946	GigaCap CM-650M, 75 µm	100 mL
0021947	GigaCap CM-650M, 75 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

PROCESS DEVELOPMENT ORDERING INFORMATION BULK MEDIA

ORDERING INFORMATION

Part #	Description	Container size
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
D. Mixed-Mode		
TOYOPEARL bulk resins		
0022817	MX-Trp-650M, 75 µm	25 mL
0022818	MX-Trp--650M, 75 µm	100 mL
0045045	Ca^{++}Pure-HA	50 g
0045039	Ca^{++}Pure-HA	100 g
E. Hydrophobic Interaction Chromatography		
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		
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
0021301	PPG-600M, 65 µm	25 mL
0021302	PPG-600M, 65 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
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
0007477	Butyl-650M, 65 µm	100 mL
0043127	Butyl-650C, 100 µm	25 mL
0007478	Butyl-650C, 100 µm	100 mL
0019955	SuperButyl-550C, 100 µm	25 mL
0019956	SuperButyl-550C, 100 µm	100 mL
0019802	Butyl-650M, 65 µm	25 mL
0044465	Hexyl-650C, 100 µm	25 mL
0019026	Hexyl-650C, 100 µm	100 mL
F. Affinity Chromatography		
TSKgel bulk resins		
0016208	Tresyl-5PW (10)	2 g*
TOYOPEARL bulk resins		
0023486	AF-rProtein L-650F, 45 µm	10 mL
0023487	AF-rProtein L-650F, 45 µm	25 mL
0023488	AF-rProtein L-650F, 45 µm	100 mL
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
0043412	AF-Carboxy-650M, 65 µm	10 mL
0008006	AF-Carboxy-650M, 65 µm	25 mL
0008041	AF-Carboxy-650M, 65 µm	100 mL
0043413	AF-Formyl-650M, 65 µm	10 mL
0008004	AF-Formyl-650M, 65 µm	25 mL
0008040	AF-Formyl-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*
0014471	AF-Tresyl-650M, 65 µm	5 g*
0014472	AF-Tresyl-650M, 65 µm	100 g*
0014475	AF-Chelate-650M, 65 µm	25 mL
0019800	AF-Chelate-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

**1 g is approximately 3.5 mL*

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 G4000SWXL. 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 188.

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 A

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, please contact our Technical Service Specialists at +49 (0) 6155 7043736.

CLEANING SOLUTIONS
SIZE EXCLUSION, TSKgel SW AND SWXL 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 PWXL 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 250µL increments of solutions 2 & 3 on analytical columns. Inject proportionally larger volumes on semi-preparative columns.*

APPENDIX B

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).

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

FIGURE 2
GUARD CARTRIDGES

FIGURE 3
GUARD CARTRIDGE HOLDER

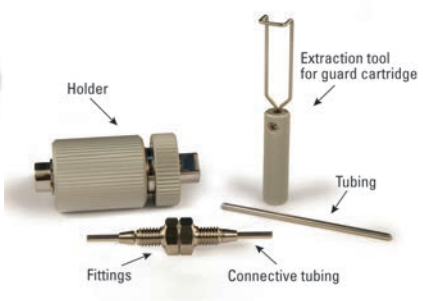


FIGURE 4
GUARDFILTER

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 Technical Service.

APPENDIX B

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:

$$(CV)pc / (CV)ac = (mg\ product)pc / (mg\ product)ac$$

in which pc and ac refer to the preparative and analytical column respectively. The volume of a column is equal to 1/4 π (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 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 or superficially porous silica particles 1.5 to 10 µm in diameter, or a monolithic rod.
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

L18- Amino and cyano groups chemically bonded to porous silica particles, 3 - 10 µm in diameter.
Recommendations: TSKgel CN-80Ts, NH_2-100
See: Reversed Phase/HILIC section

L21 - A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30 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, 5 - 15 µm in diameter
Recommendations: TSKgel SCX
See: Ion Exchange section

L23 - An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, 7 to 12 µm in size
Recommendations: TSKgel SuperQ-5PW, BioAssist Q, Q-STAT, and DNA-STAT
See: Ion Exchange 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

APPENDIX C

UNITED STATES PHARMACOPEIA (USP) SPECIFICATIONS AND CORRESPONDING TOSOH BIOSCIENCE COLUMNS

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 G3000PW$_{XL}$, G3000PW, G3000PW$_{XL}$-CP
See: Size Exclusion section

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 or sulfoethyl groups, 1 to 10 µm in diameter.
Recommendations: TSKgel SP-2SW
See: Ion Exchange section

L58- Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30 µm diameter.
Recommendations: TSKgel SCX (Na^+)
See: Ion Exchange section

L59- Packing for the size-exclusion separations of proteins (separation by molecular weight) over the range of 5 to 7000 kDa. The packing is spherical 1.5 - 10 µm, silica or hybrid packing with a hydrophilic coating.
Recommendations: TSKgel UP-SW series, SuperSW series; UltraSW series; SW$_{XL}$ and SW series
See: Size Exclusion section

L67 - Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10 µm in diameter.
Recommendations: TSKgel Octadecyl-2PW/-4PW
See: Reversed Phase 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

L89 - Packing having the capacity to separate compounds with a molecular weight range from 100 - 3000 (as determined by polyethylene oxide), applied to neutral and anionic watersoluble polymers.
Recommendations: TSKgel G-Oligo-PW, SuperOligoPW
See: SEC section

INDEX

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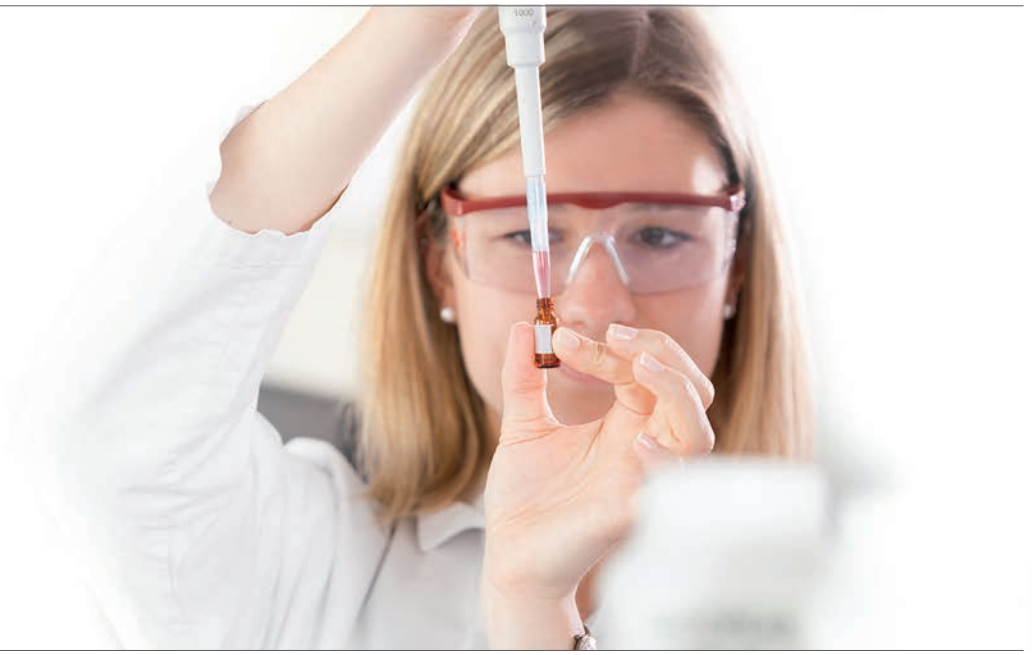

ANY QUESTIONS?

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

LOOKING FOR INSTRUCTION MANUALS OR APPLICATION NOTES?

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