

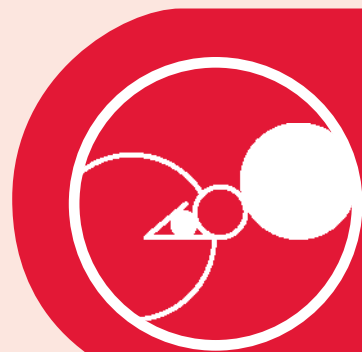
Size Exclusion Chromatography

TSKgel SW-type
SW
SW_{XL}
Super SW

TSKgel PW-type
PW
PW_{XL}

TSKgel Alpha-type
Alpha
SuperAW

TSKgel H-type
H_{XL}
H_{HR}
SuperH
SuperHZ



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Introduction to TSK-GEL Size Exclusion Chromatography Columns



Tosoh Bioscience provides TSK-GEL columns for both modes of Size Exclusion Chromatography (SEC), which are Gel Filtration Chromatography (GFC) and Gel Permeation Chromatography (GPC). GFC refers to the SEC separation of water soluble polymers in aqueous mobile phases, while GPC refers to the SEC separation of organic soluble polymers using an organic solvent as mobile phase. From a sample perspective, the TSK-GEL SW-type column line, which is based on spherical porous silica particles, is most suitable for analyzing proteins and peptides by gel filtration. Polymer-based TSK-GEL PW columns are best used for GFC analysis of other water soluble polymers, such as oligosaccharides, acrylic acid, etc. Polymer-based TSK-GEL Alpha and SuperAW columns can be used in aqueous solvents, and also with polar organic solvents, thus bridging the gap between GFC and GPC. Organic soluble polymers are best separated by GPC using polystyrene/divinylbenzene-based TSK-GEL H_{XL}, H_{HR}, SuperH or SuperHZ columns. *Table I* compares the characteristics of the various TSK-GEL column lines for SEC.

Tosoh Corporation has a proud history of innovation in size exclusion chromatography. The porous silica-based SW columns were originally introduced in 1978 as TSKgel G2000SW, G3000SW and G4000SW, using 10 and 13µm spherical particles. The second generation of SEC columns for biopolymer analysis was introduced in 1987. By reducing the particle size from 10 to 5µm for G2000SW_{XL} and G3000SW_{XL}, and from 13 to 8 µm for G4000SW_{XL}, analysis times were cut in half without sacrificing resolution. The third generation of SW-type columns was introduced in 1998 under the name SuperSW, a column line that features 4µm spherical particles packed in 4.6mm ID columns rather than the wider (7.8mm ID) SW_{XL} columns. TSK-GEL SuperSW columns are available in two pore sizes that mimic the pore structure of the G3000SW_{XL} and G2000SW_{XL} columns. Due to their higher column efficiency and smaller column volume, these columns provide better resolution and higher sensitivity in sample limited cases. This trend towards smaller, more efficient particles packed into narrower bore columns has been expanded to include columns for the analysis of non-biological aqueous and non aqueous polymers. SuperAW, SuperH and SuperHZ columns represent the state of the art column technology for high throughput SEC analysis.

Bulk polymeric Toyopearl GFC resins for process scale separations are available in convenient LABPAK samplers (up to 150mL) and in bulk quantities of up to 500mL (see the Bulk Resins

section of this catalog). For larger volumes of Toyopearl GFC media, please request a copy of the process media catalog.

Column Selection

The complete TSK-GEL SW, PW, Alpha and SuperAW column lines for GFC consist of many packings, available in various pore and particle sizes. The main criterion in choosing between the TSK-GEL SW, PW, Alpha and SuperAW SEC columns is the molecular weight of the sample and its solubility. The fact that the TSK-GEL SW columns are based on silica and the TSK-GEL PW, Alpha and SuperAW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences. See the Molecular Weight Range Tables in each column type section to determine the best column choice based on pore size.

Due to their higher resolving power, the TSK-GEL SW columns are suitable for the separation of monodisperse biopolymers such as proteins and nucleic acids. TSK-GEL PW columns are commonly used for the separation of synthetic water-soluble polymers because they exhibit a much larger separation range, better linearity of calibration curves, and less adsorption than the TSK-GEL SW columns. While a TSK-GEL SW column is typically the first column to try for biopolymers, TSK-GEL PW columns have demonstrated good results for smaller peptides (<1,000Da), protein aggregates, DNA fragments, and viruses.

The TSK-GEL Alpha Series columns offer a new alternative for performing SEC. Their compatibility with a wide range of solvents makes them useful for both gel filtration chromatography (GFC) and gel permeation chromatography (GPC). TSK-GEL SuperAW columns are based upon the the same chemistry as Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high-throughput applications. Unlike the Alpha columns, mixed bed formats are included in the SuperAW product offerings for samples with wide ranges of molecular weights/hydrodynamic radius. See *Figure 1* for more information on compatibility of all TSK-GEL SEC columns and solvents.

Non-aqueous GPC separations are performed with TSK-GEL H-type pre-packed columns. Our TSKgel MultiporeH_{XL}-M column contains porous polystyrene divinylbenzene packings with various sized pores on a single bead. TSK-GEL SuperH and SuperHZ columns are offered for high throughput applications where reductions in run time and solvent consumption are critical. *Table XII* (see p. 61) provides a list of possible shipping solvents along with compatible solvents for each H-type packing.

See *Table II* for suggestions on how to choose between TSK-GEL SW, TSK-GEL PW and TSK-GEL Alpha column types.

Features	Benefits
Rigid hydrophilic packings	Minimal swelling and excellent physical strength Low adsorption resulting in high mass recovery
Four series of SEC columns with different ranges of solvent compatibility (<i>Figure 1</i>)	Suitable for aqueous GFC and non-aqueous GPC
Easy scale up	Analytical and preparative pre-packed SEC columns

Table 1

Characteristics of TSK-GEL Size Exclusion Column Lines				
Column Line	TSK-GEL SW	TSK-GEL PW	TSK-GEL Alpha/ SuperAW	TSK-GEL H
Resin Type	Silica	Methacrylate	Methacrylate	PS-DVB
No. of Available Pore Sizes	3	7	5	8
pH stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0	1.0 - 14.0
Solvent Compatibility	100% polar	50% polar	100% polar and nonpolar	100% nonpolar, limited polar
Max. Temperature	30°C	80°C*	80°C	60°C (1000H-3000H) 80°C (4000H-GMH) 140°C (H _{HR} , H- HT)
Max. Flow Rate (mL/min)	0.4 (SuperSW) 1.2 (SW and SW _{XL})	1.0 (PW _{XL}) 1.2 (PW)	1.0	1.0 (H _{HR}) 1.2 (H _{XL}) 0.8 (SuperH) 0.7 (SuperHZ 6.0mmID) 0.4 (SuperHZ 4.6mmID)
Pressure** (kg/cm ²)	8-120	10-40	20-40	10-60
Application Focus	proteins	water-soluble polymers	intermediate polar polymers	organic-soluble polymers

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

** Depends on column dimensions and particle size

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

Figure 1

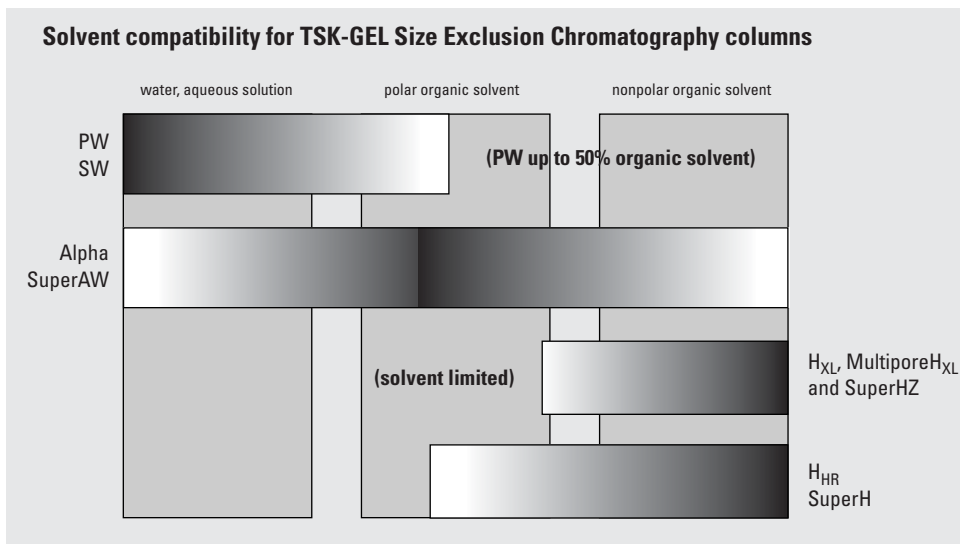


Table II

Column selection guide for high performance Gel Filtration Chromatography						
<i>Sample</i>		<i>Column selection</i>			<i>Selection criteria</i>	
		<i>First choice</i>		<i>Alternative</i>		
Carbo-hydrates	polysaccharides	TSKgel GMPW _{XL}		G5000PW _{XL} and G3000PW _{XL}	large pore size, linear calibration curve, small particles, high resolving power	
	oligosaccharides	TSKgel G-Oligo-PW or TSKgel G2000PW		G2500PW _{XL}	small particles, high resolving power	
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PW _{XL}		large pore size, small particles, high resolving power	
		medium and small	TSKgel G4000SW _{XL} / SW/ TSKgel BioAssist G4SW _{XL} , TSKgel Super SW3000, or G3000SW _{XL} / SW/ TSKgel BioAssist G3SW _{XL}		suitable pore sizes	
	RNA	TSKgel G4000SW _{XL} / SW/ TSKgel BioAssist G4SW _{XL} , TSKgel Super SW3000, or G3000SW _{XL} / SW/ TSKgel BioAssist G3SW _{XL}		suitable pore sizes		
	oligonucleotides	TSKgel G2500PW _{XL}		—	small pore size, ionic interaction	
Proteins	normal size small-medium proteins	TSKgel Super SW3000, G3000SW _{XL} / SW/ TSKgel BioAssist G3SW _{XL} , TSKgel G4000SW _{XL} / SW, TSKgel BioAssist G4SW _{XL} , TSKgel Super SW2000, or G2000SW _{XL} / SW/ TSKgel BioAssist G2SW _{XL}		G3000PW _{XL} or G4000PW _{XL}	small particles small to medium range pore sizes	
	large proteins	low density lipoprotein	TSKgel G6000PW _{XL} or TSKgel G5000PW _{XL}		—	large pore sizes
		gelatin	TSKgel GMPW _{XL}		G5000PW _{XL} and G3000PW _{XL}	large pore size, linear calibration curve
Peptides	large	TSKgel Super SW3000, G3000SW _{XL} / SW/ TSKgel BioAssist G3SW _{XL} or G2000SW _{XL} / SW/ TSKgel BioAssist G2SW _{XL}		Super SW2000 or G3000PW _{XL}	small to medium range pore size, versatile	
	small	TSKgel G2500PW _{XL}		Super SW2000 or G2000SW _{XL} / SW	linear calibration curve, high resolving power	
Viruses		TSKgel G6000PW _{XL} or TSKgel G5000PW _{XL}		—	large pore size, high resolving power	
Synthetic polymers		TSKgel GMPW _{XL} or TSKgel Alpha-M		G5000PW and G3000PW _{XL} or Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve	
Synthetic oligomers	nonionic and cationic	TSKgel G-Oligo-PW, TSKgel G2500PW _{XL} or TSKgel Alpha-2500		G2500PW or Super AW2500	small pore size, high resolving power	
	anionic	TSKgel G2500PW _{XL} or TSKgel Alpha-2500		G2500PW or Super AW2500	small pore size, ionic interaction	

Applications

Polynucleotides

TSKgel G2000SW, G3000SW, G4000SW, and G5000PW columns are effective in separating double-stranded DNA fragments and ribosomal and transfer RNA. The choice of column is dependent on sample molecular weight. Small nucleic acids are adequately analyzed by using TSK-GEL SW columns. Larger nucleic acids should be analyzed with TSK-GEL PW columns of larger pore size, such as the TSKgel G-DNA-PW and TSKgel G5000PW columns. Calibration curves for double-stranded DNA fragments on TSK-GEL SW type columns and a TSKgel G5000PW column are shown in *Figure 2*; *Table III* lists the recommended TSK-GEL SW and TSK-GEL PW columns for separating double-stranded DNA and RNA fragments.

Separation of four *E. coli* RNAs, shown in *Figure 3*, confirms the better performance of TSK-GEL SW columns for samples with a wide molecular weight range. The sample consists of 4S tRNA (25,000Da), 5S rRNA (39,000Da), 16S rRNA (560,000Da), and 23S rRNA (1,100,000Da). All four polynucleotides are within the molecular weight range recommended for TSK-GEL SW type columns. The two chromatograms demonstrate a superior separation with the TSKgel G4000SW column.

Figure 2

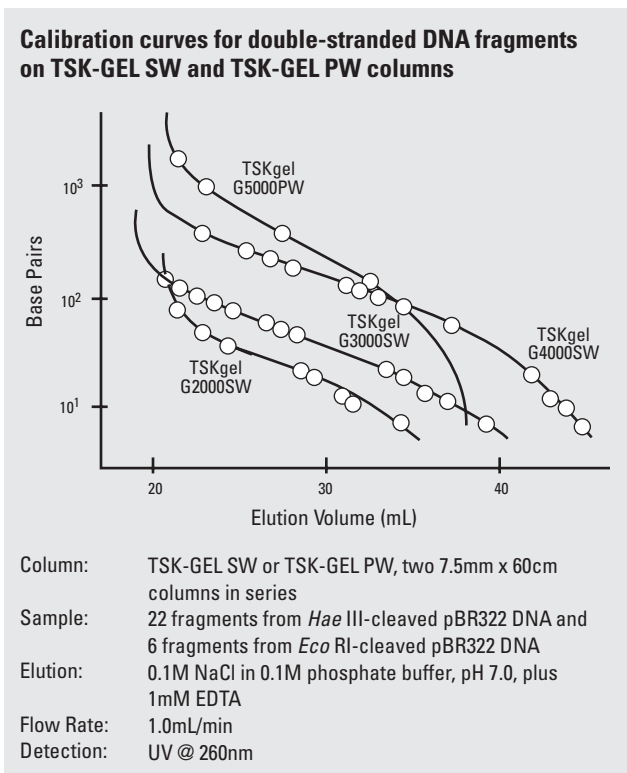


Table III

Recommended TSK-GEL SW and TSK-GEL PW columns for separating double-stranded DNA and RNA fragments

Base pairs of DNA	Recommended column
< 55	TSKgel G2000SW _{XL} /SW or G3000SW _{XL} /SW or Super SW2000 or 3000
55 – 110	TSKgel G3000SW _{XL} /SW or Super SW3000
110 – 375	TSKgel G4000SW _{XL} /SW
375 – 1500	TSKgel G5000PW _{XL} /PW
1000 – 7000	TSKgel G-DNA-PW

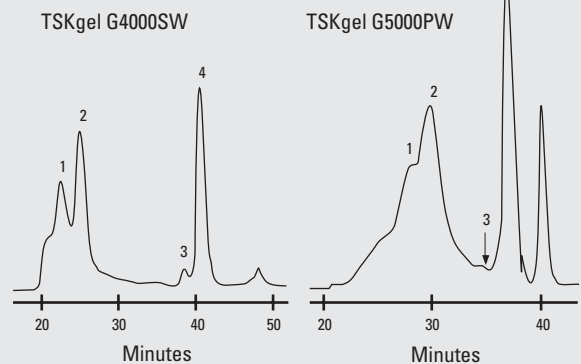
RNA (Da) Molecular Weight

< 60,000	TSKgel G2000SW _{XL} /SW or G3000SW _{XL} /SW or Super SW2000 or 3000
60,000 – 120,000	TSKgel G3000SW _{XL} /SW or Super SW3000
120,000 – 1,200,000	TSKgel G4000SW _{XL} /SW
1,200,000 – 10,000,000	TSKgel G5000PW _{XL}

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Figure 3

Separation of total *E. coli* RNA on TSK-GEL SW and TSK-GEL PW columns



Peptides

As Figure 4 shows, the calibration curves for TSKgel G2500PW_{XL} and TSKgel G2000SW_{XL} are very similar for samples below 3,000Da. The data was generated using 17 samples ranging in size from myoglobin (17,800Da) to glycine (75Da). While the curves are similar in shape through this range of sample sizes, each sample molecule behaved differently on the two columns, indicating additional sample-resin interaction. For example, although an organic solvent was used to reduce hydrophobic effects, the elution of hydrophobic peptide leu-enkephalin was delayed on the TSKgel G2500PW_{XL} column.

Small peptides may be difficult to chromatograph by aqueous GFC, due to complex non-size effects such as ionic and hydrophobic interactions. The addition of organic solvents and

buffered salt solutions overcome these effects. Figure 5 compares the separation of two mixtures of peptides on both TSKgel G2500PW_{XL} and TSKgel G2000SW_{XL} columns to demonstrate which might be superior for a particular type of peptide. The first group of peptides had molecular weights ranging from 6,500Da to 555Da. In the second group, the range was from 75Da to 17,800Da. The chromatograms confirm that TSKgel G2000SW_{XL} columns give higher resolution for most peptide mixtures, but do not perform as well as TSKgel G2500PW_{XL} columns at peptide molecular weights lower than 1,000Da. For very small peptides, the TSK-GEL PW_{XL} column type is preferable.

Figure 4

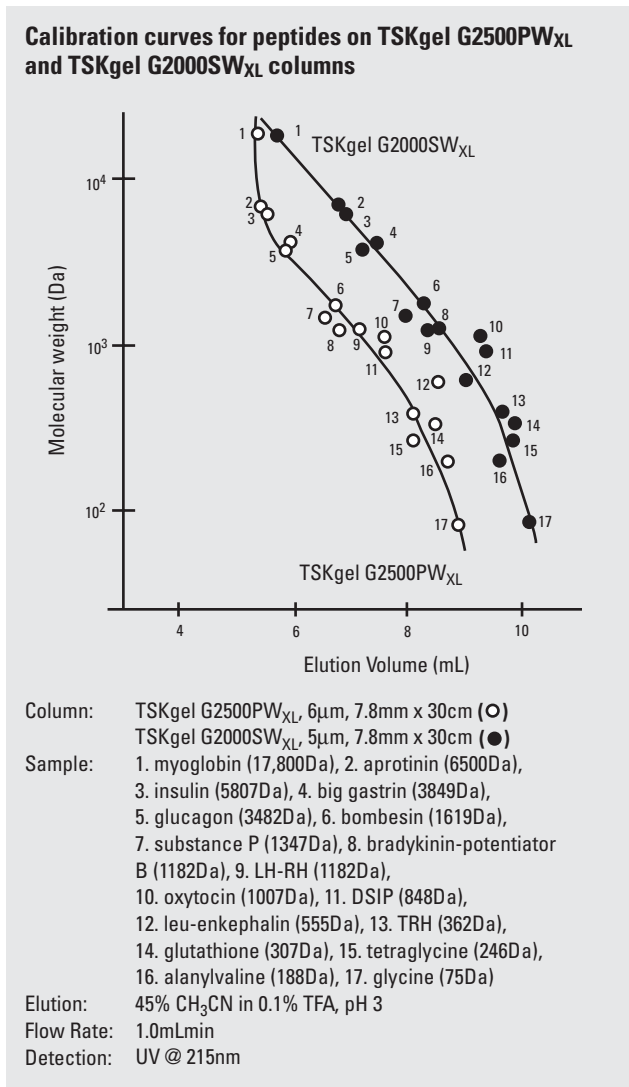
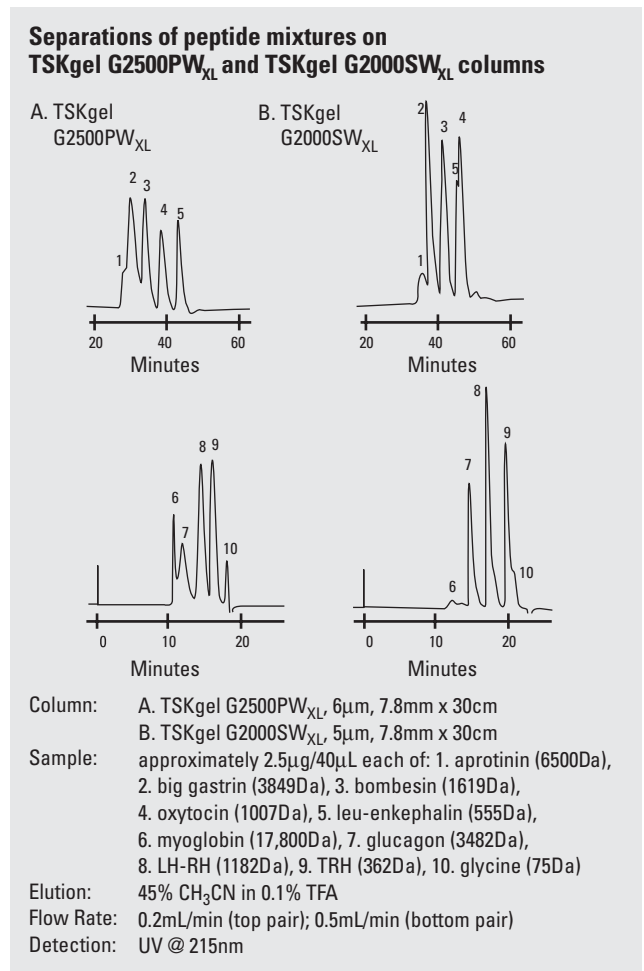


Figure 5



Proteins

In general, TSK-GEL SW columns have a fractionation range that matches the molecular weight range of most proteins and peptides. However, analytical methods that utilize combinations of TSK-GEL SW and TSK-GEL PW columns in series for isolating large lipoproteins (high and low density), chylomicron, etc. are illustrated in *Figure 6*.

As shown in *Figure 7*, successful elution of the hydrophobic amphoteric polymer collagen (a connective tissue protein) and gelatin on the TSKgel GMPW column requires the addition of 20% CH₃CN to 0.1M NaNO₃. Peak areas are reduced and elution is not reproducible when the organic solvent is omitted from the elution buffer for hydrophobic samples.

IgG

The most suitable column and mobile phase will depend on the particular components that need to be measured. TSK-GEL SW_{XL} columns can provide fast, simplified quality control analyses of proteins, peptides and other large-size biopharmaceuticals. For example, a therapeutic solution of intravenous IgG may contain albumin as a stabilizer, and both proteins must be quantified following manufacture. Although literature reports describe the separation of these two proteins by many other chromatographic methods, long analysis times and complex gradient elutions are required. A method developed on TSKgel G3000SW_{XL} provides quantitative separation of the two proteins in 15 minutes with a simple, isocratic elution system. As shown in *Figure 8*, human albumin can be separated from a 20-fold excess of IgG, and quantified using an optimized elution buffer. This simple separation method can be applied to the isolation of other IgGs, such as monoclonal antibodies in ascites fluid.

Figure 6

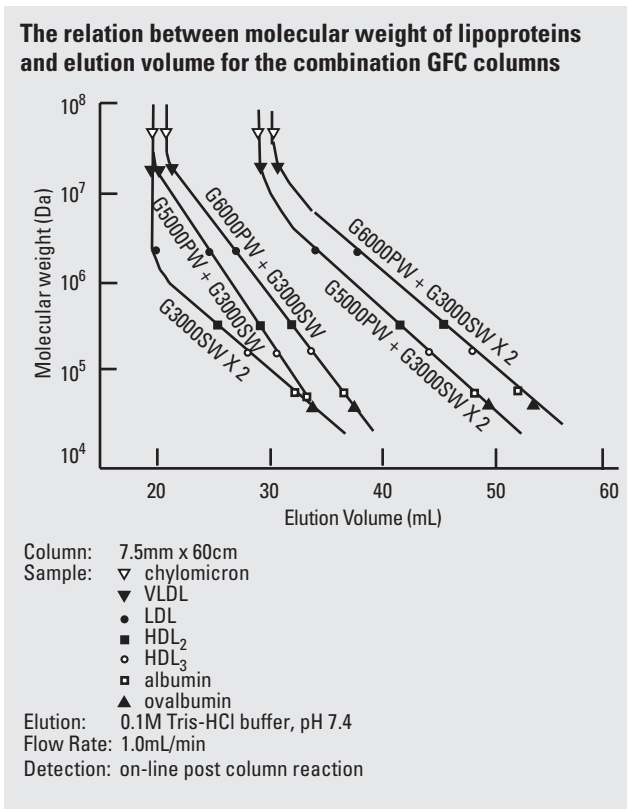


Figure 7

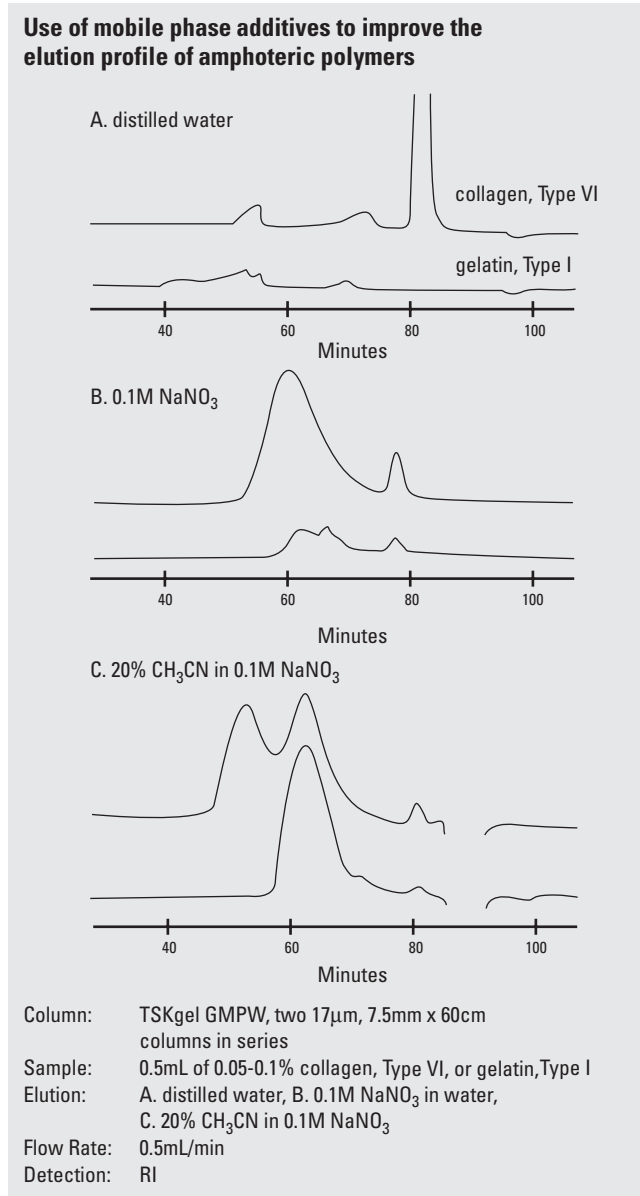
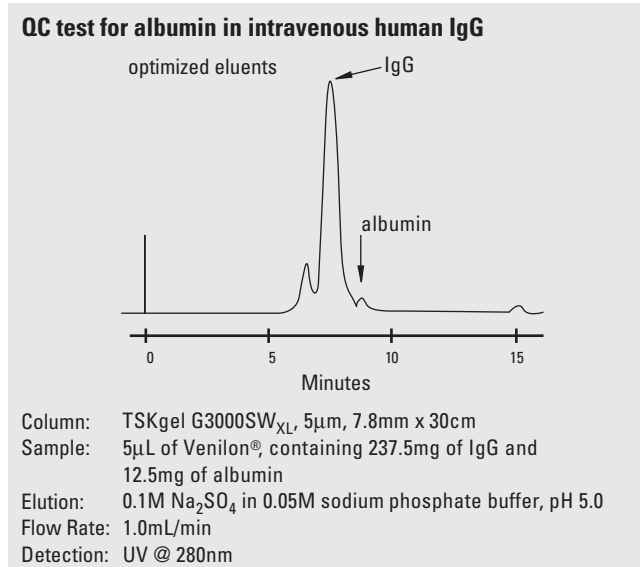


Figure 8





Cleaning Procedures

Please see Appendix A for instructions on how to maintain and clean TSK-GEL Size Exclusion columns.

Improving resolution with SEC columns

- Verify that the column is not overloaded (refer to sample load information in the SW column section).
- Decrease the dead volume in the HPLC system by using the shortest tubing lengths and the smallest tubing ID possible without exceeding the maximum pressure for the column.
- Decrease the flow rate, but not lower than 0.3mL/min for TSK-GEL SW and SW_{XL} columns because increased diffusion will occur.

Note: TSK-GEL Super SW columns show optimum resolution at flow rates below 0.3mL/min because of their small diameter.

- If using a 30cm column, add an additional 30cm column or switch to a TSK-GEL_{XL}-type column that has a smaller particle size. Resolution will improve more than two-fold. Alternatively, if using the SW_{XL}-type columns, switch to the TSK-GEL Super SW that offer increased sensitivity and resolution due to 50% more theoretical plates.

Literature

For additional information describing applications of Size Exclusion Chromatography columns, or how to select the optimal Size Exclusion Chromatography column, please contact our Technical Service specialists at 1-800-366-4875 (option 3) or refer to Tosoh HPLC database on our website: www.tosohbioscience.com.