

Introduction of Aqueous SEC Columns: TSKgel PW_{XL} series

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1. Introduction

Column technology, as it applies to high performance gel filtration chromatography (often referred to as GFC, aqueous size exclusion chromatography or aqueous gel permeation chromatography), has made remarkable progress. Several excellent reviews¹⁻⁷ have been published.

TSK-GEL PW-type columns have clearly been one of the leading products in this field. Many papers on polymer characterization⁸⁻¹³ and applications of TSK-GEL PW-type columns have been published. Representative examples of important applications include such biopolymers as polysaccharides^{8,11,13-19}, polynucleotides^{20,21}, large proteins^{14,22-31}, peptides^{32,33}, synthetic water-soluble polymers^{8,13,14,34-37} and oligomers^{2,13,38-44}.

A new series of TSK-GEL PW-type columns, consisting of six TSK-GEL PW_{XL} columns and two special columns (TSKgel G-Oligo-PW and TSKgel G-DNA-PW), has been introduced into the market with drastically increased resolution and a reduction in analysis time. In addition, two new grades have been added to enlarge the application range of the TSK-GEL PW-type product line. Main features and improvements of the new series are summarized as follows in comparison with the conventional series:

(1) Higher performance

The number of theoretical plates (per unit column length) of the new TSK-GEL PW_{XL} series columns is practically more than double that of the conventional series columns. Therefore the resolving power of the new TSK-GEL PW_{XL} series columns is about 1.4 times that of the conventional series column of the same length. Compared with the conventional columns (60cm), using the new TSK-GEL PW_{XL} series columns (30cm) reduces analysis time by up to 50% without sacrificing resolution.

(2) Introduction of TSK-GEL GMPW_{XL} columns

TSK-GEL GMPW_{XL} is a new line of columns that feature excellent linearity of the calibration curve over a very wide range of molecular weight from 5×10^2 to more than 1×10^7 .

(3) Introduction of TSK-GEL G2500PW_{XL} columns

One of the disadvantages of the current TSK-GEL PW-type columns is the difference in chemical nature between the small pore size grades (TSK-GEL G1000PW and G2000PW) and the large pore size grades (TSK-GEL G3000PW~G6000PW). TSK-GEL G1000PW and G2000PW columns contain a considerable number of ionic groups (both cationic and anionic), while TSK-GEL G3000PW~G6000PW columns contain only a small number of weakly anionic groups. Therefore it is not recommended to use a TSKgel G2000PW or a TSKgel G1000PW column in conjunction with columns of larger pore size grades. To improve this situation, TSK-GEL

G2500PW grade is introduced in both the conventional series and the new PW_{XL} series.

TSK-GEL G2500PW grade has almost the same chemical nature as the larger pore size grades and it can be used in conjunction with them. Furthermore, a TSKgel G2500PW column has almost the same calibration curve as a TSKgel G2000PW column, but it should be noted that the TSKgel G2500PW column is to some degree inferior to a TSKgel G2000PW column for the separation of small molecules.

(4) Introduction of TSKgel G-Oligo-PW column

In order to further improve the resolution for oligomers, TSKgel G-Oligo-PW was introduced as a special column dedicated to the separation of non-ionic and cationic oligomers such as oligosaccharide, polyethylene glycol, etc. The packing material in a TSKgel G-Oligo-PW column contains cationic groups just as that of a TSKgel G2000PW column. Therefore the TSKgel G-Oligo-PW column is not recommended for the analysis of anionic polymers.

(5) Introduction of TSKgel G-DNA-PW column

TSKgel G-DNA-PW is a new column dedicated to the separation of large polynucleotides (for example, DNA fragments of 500~5000 base pairs). The TSKgel G-DNA-PW column, which features a very large pore size (ca. 4000Å) and a small particle size (10µm), can almost completely separate large DNA fragments that differ in size by a factor of two within 2~4 hours.

In this Separation Report the fundamental characteristics and properties of the new TSK-GEL PW-type series will be described, together with a brief review on how to select the appropriate column. The following information is described in detail: (1) separation of water-soluble oligomers on new TSK-GEL PW_{XL} columns, (2) separation of water-soluble polymers on new TSK-GEL PW_{XL} columns, (3) and separation of large DNA fragments on TSKgel G-DNA-PW column.

2. Characteristics of TSK-GEL PW_{XL} columns

Table 1 lists the new series columns consisting of six TSK-GEL PW_{XL} grades, one TSKgel G-Oligo-PW and one TSKgel G-DNA-PW grade. Table 1 also lists exclusion limits measured with standard polymers (polyethylene oxide, dextran, and proteins) and the guaranteed numbers of theoretical plates per column measured with ethylene glycol using a RI detector.

Table 2 shows the separation ranges of the TSK-GEL PW_{XL} series columns for the PEG and PEO standards.

All columns have the same column dimensions of 7.8mm inner diameter and 30cm length.

As the TSK-GEL PW_{XL} columns contain smaller particles, the guaranteed number of theoretical plates per unit length is more than 2.8 times compared with those of the corresponding traditional TSK-GEL PW columns, as is shown in Table 3.

Table 1 Characteristics of the New Series of TSK-GEL PW-type Columns

Column	Particle Size µm	Minimum Number Theoretical Plates ¹ (TP/Column)	Exclusion Limit ²			Column Dimension mm ID × cm
			PEO	Dextran	Protein	
TSKgel G2500PW _{XL}	7	14,000	5×10 ³			7.8×30
TSKgel G3000PW _{XL}	7	14,000	8×10 ⁴	2×10 ⁵	8×10 ⁵	
TSKgel G4000PW _{XL}	10	10,000	4×10 ⁵	1×10 ⁶	(>4×10 ⁶)	
TSKgel G5000PW _{XL}	10	10,000	1×10 ⁶	(>2.5×10 ⁶)	(>1×10 ⁷)	
TSKgel G6000PW _{XL}	13	7,000	(2×10 ⁷)	(>5×10 ⁷)	(>2×10 ⁸)	
TSKgel GMPW _{XL}	13	7,000	(2×10 ⁷)	(>5×10 ⁷)	(>2×10 ⁸)	
TSKgel G-Oligo-PW	7	14,000	5×10 ³			7.8×30
TSKgel G-DNA-PW	10	10,000	2×10 ⁷			

¹Measurement conditions for theoretical plate number.

Eluent: distilled water

Flow rate: 1.0mL/min

Sample: 20µL ethylene glycol 1%

²Values in parentheses are estimates.

Table 2 Separation Range of the New Series of TSK-GEL PW-type Columns for PEG and PEO Standards

Column	Separation Range (dalton)
TSKgel G2500PW _{XL}	< 3,000
TSKgel G3000PW _{XL}	< 40,000
TSKgel G4000PW _{XL}	2,000 - 300,000
TSKgel G5000PW _{XL}	4,000 - 800,000
TSKgel G6000PW _{XL}	40,000 - 8,000,000
TSKgel GMPW _{XL}	1,000 - 8,000,000
TSKgel G-Oligo-PW	< 3,000
TSKgel G-DNA-PW	40,000 - 8,000,000

Table 3 Comparison of the Minimum Number of Theoretical Plates for TSK-GEL PW and PW_{XL} Series Columns

Grade	TSK-GEL PW Series		TSK-GEL PW _{XL} Series
	7.5mm ID × 60cm	7.5mm ID × 30cm	7.8mm ID × 30cm
TSKgel G2500PW	10,000/column	5,000/column	14,000/column
TSKgel G3000PW	10,000	5,000	14,000
TSKgel G4000PW	6,000	3,000	10,000
TSKgel G5000PW	6,000	3,000	10,000
TSKgel G6000PW	6,000	3,000	7,000
TSKgel GMPW	6,000	3,000	7,000

Figures 1 to 3 show calibration curves for TSK-GEL PW_{XL} columns measured with PEG, PEO, dextran, and protein standards

In Figure 4 the calibration curve for a TSKgel G-Oligo-PW column (solid line) together with the one for a TSKgel G2500PW_{XL} column (dotted line) measured with polyethylene glycol standards is shown. The calibration curve of a TSKgel G-DNA-PW column for double-stranded DNA fragments is presented elsewhere⁴².

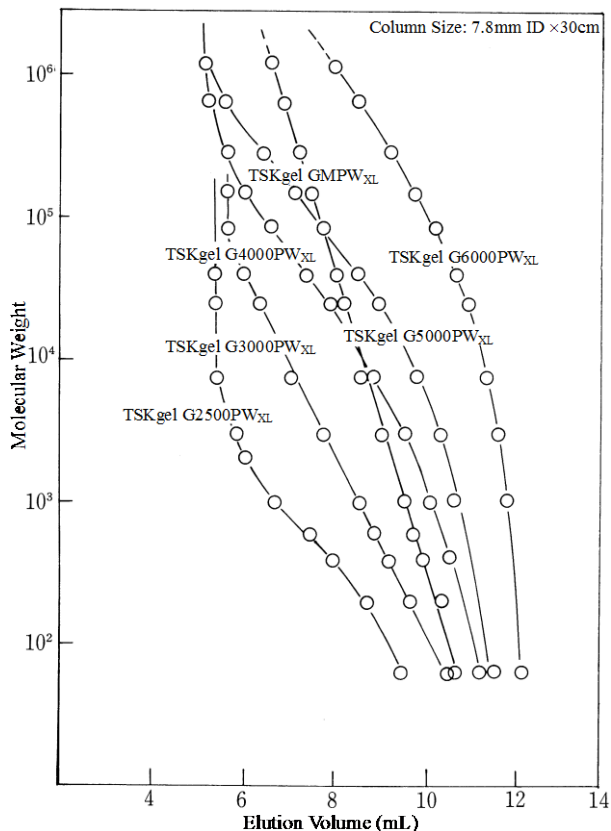


Fig. 1 Calibration Curves for TSK-GEL PW_{XL} Columns for PEG and PEO Standards

Sample: PEG and PEO Standards

Eluent: distilled water

Flow rate: 1.0mL/min

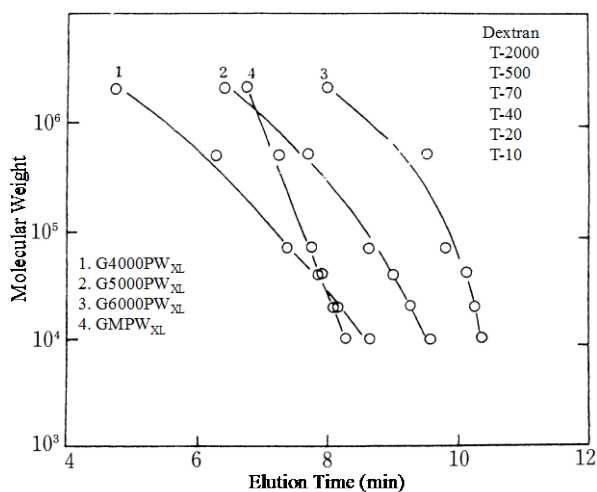


Fig. 2 Calibration Curves for TSK-GEL PW_{XL} Columns for Dextran Standards

Eluent: 0.2M phosphate.buffer (pH6.8)

Flow rate: 1.0mL/min

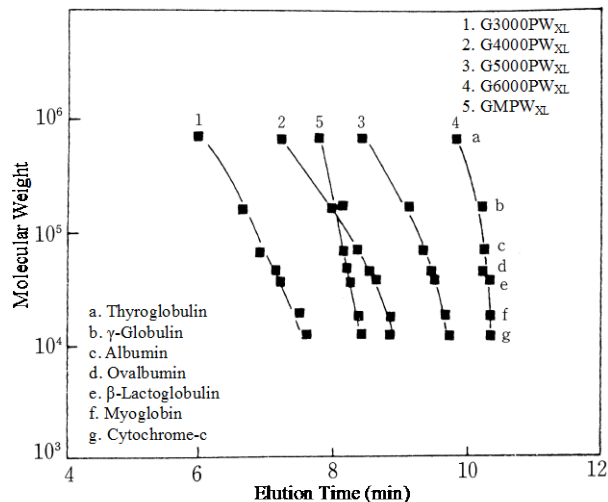


Fig. 3 Protein Calibration Curves for TSK-GEL PW_{XL} Columns

Eluent: 0.2 mol/L phosphate.buffer (pH6.8)

Flow rate: 1.0mL/min

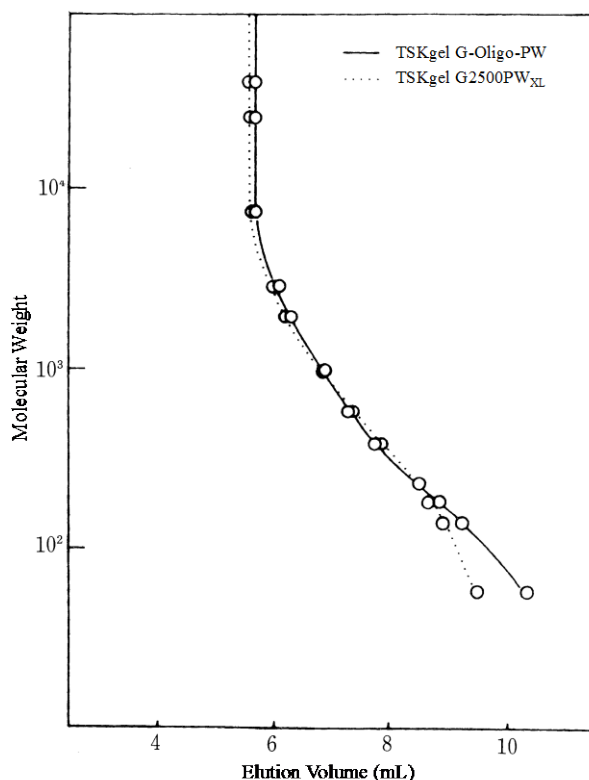


Fig. 4 Calibration Curves for TSK-GEL G-Oligo-PW and TSK-GEL G2500PW_{XL} columns

Column size: 7.8mm ID x 30cm

Sample: PEG and PEO Standards

Eluent: distilled water

Flow rate: 1.0mL/min

3. Basic properties of TSK-GEL PW_{XL} columns

3-1. Effect of flow rate on the number of theoretical plates

The effect of flow rate on the number of theoretical plates depends on the particle size of packing material, molecular size of a sample, viscosity and temperature of the eluent, etc. For example, Fig. 5 shows how the number of theoretical plates varies with flow rate as measured with ethylene glycol (a typical small molecule) on a TSKgel G2500PW_{XL} column (6 μ m, the smallest particle size among TSK-GEL PW_{XL} series columns) against that measured with a PEO standard (a common large molecule) on a TSKgel G6000PW_{XL} column (13 μ m, the largest particle size among TSK-GEL PW_{XL} series columns). The number of theoretical plates for a small MW compound on the 6 μ m particle size column is almost constant, while that for a 25,000 dalton polymer on the 13 μ m particle size column decreases considerably as flow rate increases. Thus it is suggested to use a lower flow rate for columns with larger pore sizes when they are used for analyzing large molecules.

3-2. Ionic properties

The titration curves of the TSK-GEL PW_{XL} gels with 0.1N sodium hydroxide are shown in Figure 6. All of the TSK-GEL PW_{XL} grades contain a small number of weakly anionic groups. When using an eluent with a low ionic strength, anionic samples are excluded by ionic repulsion and as a consequence will elute earlier than expected based on their molecular size, whereas cationic samples are retained by ionic adsorption and elute later than theoretically predicted. An eluent with ionic strength of more than 0.1mol/L is generally used to eliminate such ionic interactions.

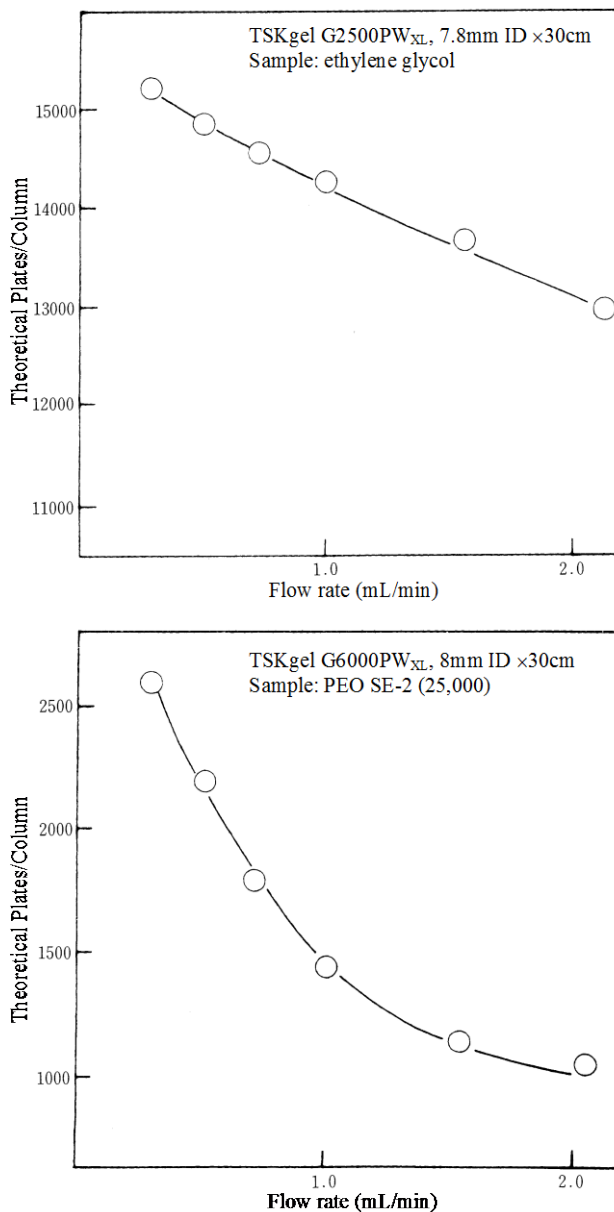


Fig. 5 Column efficiency as a function of flow rate for TSKgel G2500PW_{XL} and G6000PW_{XL} columns

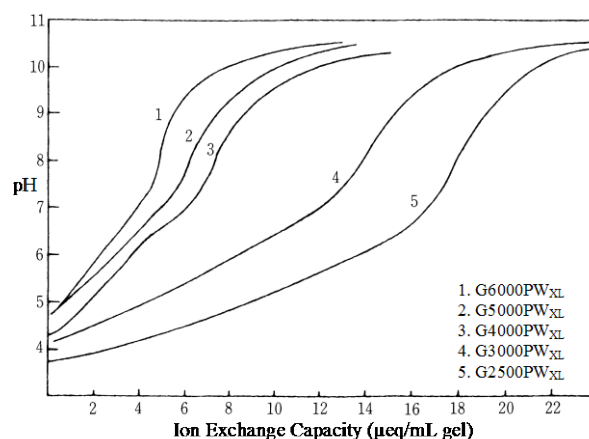


Fig. 6 Titration Curves of TSK-GEL PW_{XL} Gels

Figure 7 shows the titration curves for TSK-GEL G2000PW and G2500PW packings. It is clear that the TSKgel G2500PW column has a much reduced ion exchange capacity. The titration curve of the packing of TSKgel G-Oligo-PW is very similar to that of TSKgel G2000PW.

Figure 8 shows the effect of sodium chloride concentration on the elution volume of adenosine monophosphate (a typical anionic sample) on a TSKgel G2500PW_{XL} and G-Oligo-PW column. Clearly, adenosine monophosphate is strongly retained at decreasing NaCl concentration.

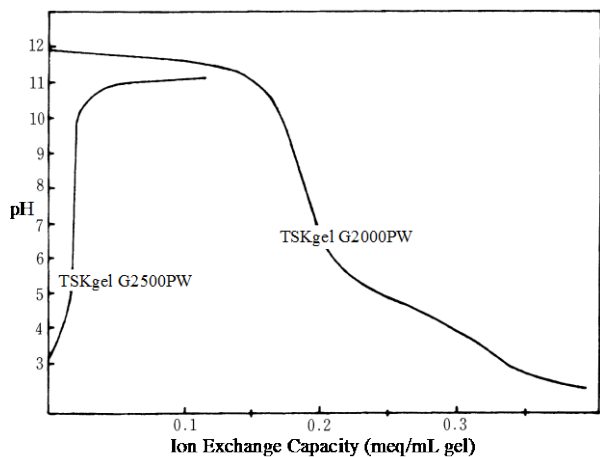


Fig. 7 Comparison of Titration Curves between TSKgel G2500PW and G2000PW Gels

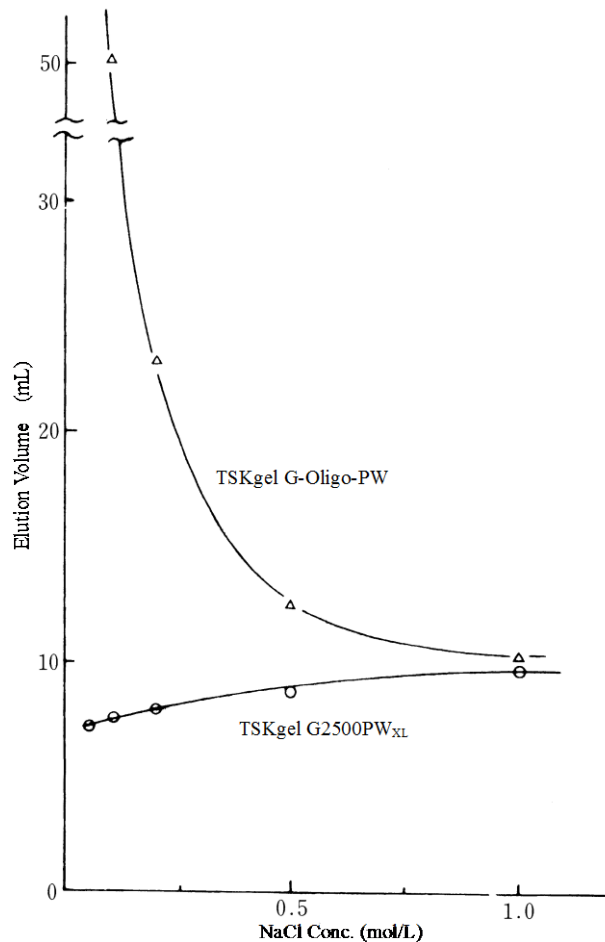


Fig. 8 Retention of Adenosine Monophosphate as a Function of Salt Concentration

Column size: 7.8mm ID × 30cm

Sample: adenosine monophosphate

Eluent: 0.02mol/L phosphate buffer (pH6.8)
+0.05mol/L-1.0mol/L NaCl

3-3. Hydrophobic property

TSK-GEL PW- and PW_{XL}-type gels are more hydrophobic than polysaccharide gels composed of crosslinked dextran. In Table 4 capacity factors of several alcohols on TSK-GEL PW_{XL} columns are listed. As expected, the longer the alkyl chain, the stronger the interaction. Also, the hydrophobic interaction increases with increasing salt concentration, while it decreases when adding an organic solvent to the eluent. The dependence of elution volume of alcohols on sodium chloride concentration is shown in Figure 9. Figure 10 indicates the dependence of elution volumes of β -phenethyl alcohol, adenine, adenosine, and tryptophan on the concentration of acetonitrile in the eluent. The analytes used in this experiment are typically small, water-soluble compounds which interact strongly with TSK-GEL PW gels. As is clear from Figure 10, the solutes elute at almost normal position at 50% acetonitrile concentration.

Hydrophobic interaction can also be reduced by increasing column temperatures, which, as is shown in Figure 11, shows how the capacity factor of β -phenethyl alcohol depends on temperature. The effect of acetonitrile concentration (0, 10, and 30%) is shown in the same Figure.

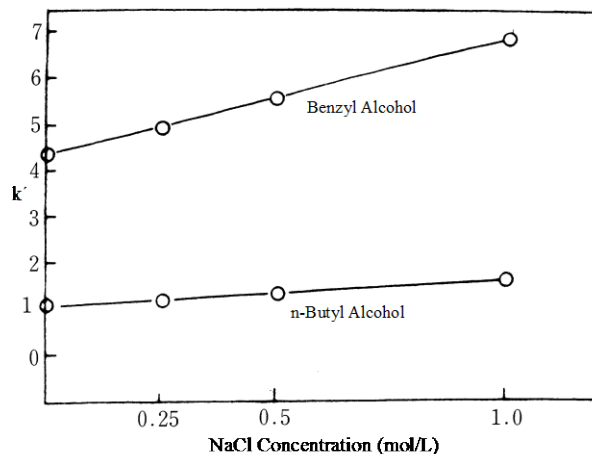


Fig. 9 Retention of Benzyl Alcohol and n-Butyl Alcohol as a Function of Sodium Chloride Concentration

Column: TSKgel G2500PW_{XL}, 7.8mm ID x 30cm

Sample: (1) benzyl alcohol

(2) n-butyl alcohol

Flow rate: 1.0mL/min

Table 4 Capacity Factors of Aliphatic Alcohols

Column	Ethyl Alcohol	iso-Propyl Alcohol	n-Butyl Alcohol	β -Phenethyl Alcohol
TSKgel G2500PW _{XL}	0.16	0.45	0.93	5.53
TSKgel G3000PW _{XL}	0.14	0.35	0.82	5.20
TSKgel G4000PW _{XL}	0.09	0.22	0.49	2.84
TSKgel G5000PW _{XL}	0.07	0.19	0.44	2.84
TSKgel G6000PW _{XL}	0.05	0.15	0.37	2.55

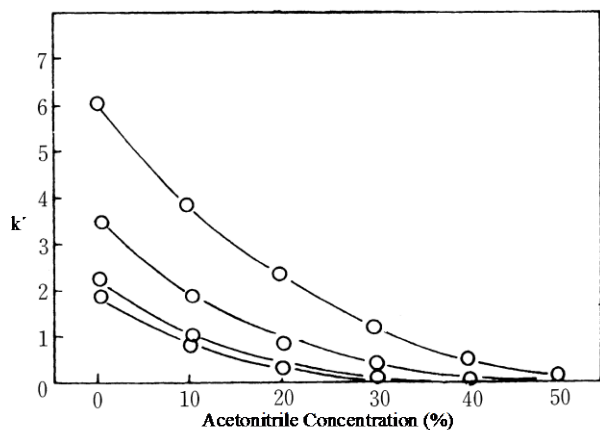


Fig. 10 Dependence of Capacity Factors of β -Phenethyl Alcohol, Adenine, Adenosine and Tryptophan on Acetonitrile Concentration

Column: TSKgel G2500PW_{XL}, 7.8mm ID \times 30cm

Sample: (a) β -phenethyl alcohol

(b) adenine

(c) adenosine

(d) tryptophan

Flow rate: 1.0mL/min

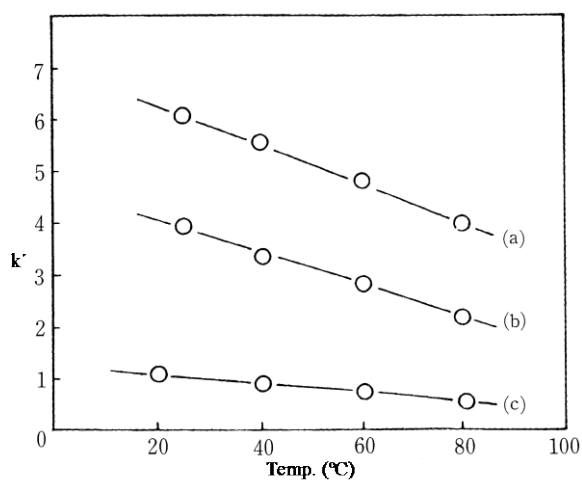


Fig. 11 Dependence of the Capacity Factor of β -Phenethyl Alcohol on Temperature

Column: TSKgel G2500PW_{XL}, 7.8mm ID \times 30cm

Sample: β -phenethyl alcohol

Eluent: (a) water

(b) 10% acetonitrile in water

(c) 30% acetonitrile in water

Flow rate: 1.0mL/min

3-4. Temperature stability

TSK-GEL PW gels are thermally stable in neutral aqueous solutions and can be autoclaved at 120°C. Columns can be used at temperatures up to 80°C in common neutral aqueous solutions. Mobile phases of high or low pH should not be used at high temperatures.

Figure 12 shows a column stability experiment for TSKgel GMPW_{XL}, TSKgel G2500PW_{XL} and TSKgel G-Oligo-PW columns at a temperature of 60°C. During a three month period, theoretical plate numbers and pressure drop did not change.

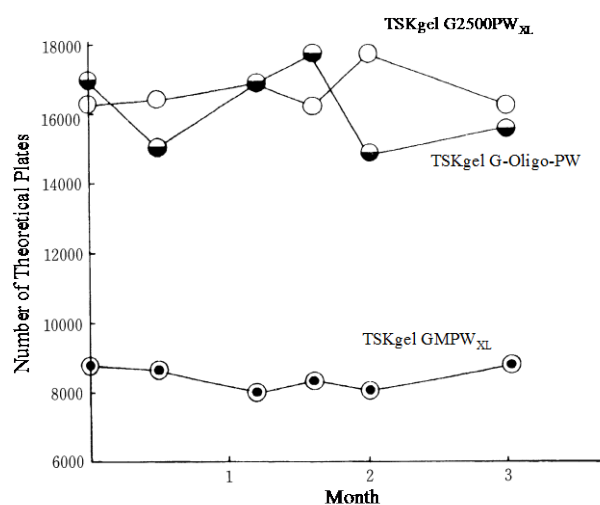


Fig. 12 An Example of Column Life Test at 60°C

Column size: 7.8mm ID \times 30cm

Sample: ethylene glycol

Running Condition:

Flow rate: 1.2mL/min.

Temp.: 60°C

Measuring Condition:

Flow rate: 1.0mL/min

Temp.: 25°C

3-5. Solvent compatibility

1) Organic solvent

Water-soluble organic solvents are frequently used as a modifier in order to suppress hydrophobic interaction between sample components and TSK-GEL PW_{XL} columns. Some routinely used examples are listed in Table 5.

All TSK-GEL PW- and PW_{XL}-type columns, except TSKgel G-DNA-PW, are compatible with mobile phases containing 20% water-soluble organic solvents, such as methanol, ethanol, isopropanol, acetonitrile, formic acid, acetic acid, dimethyl formamide, dimethyl sulfoxide, acetone, etc.

Table 5 Typical Examples of Use of Organic Solvent as Modifier

No.	Sample	TSKgel Column	Eluent	Reference
1	Peptides	G3000PW	0.1% TFA Containing 36-45% CH ₃ CN	32, 33
2	Poly(vinyl pyrrolidone)	G5000PW + G3000PW	0.1mol/L Sodium Acetate containing 20% CH ₃ CN	14
3	Poly(styrene sulfonate)	G6000PW + G3000PW	0.2mol/L Phosphate Buffer containing 10% CH ₃ CN	14
4	Poly(dimethyl aminoethyl methacrylate)	G6000PW + G3000PW	0.5mol/L Sodium Acetate containing 0.5mol/L Acetic Acid	14
5	Poly(ethyleneimine)	G6000PW + G3000PW	0.5mol/L Sodium Acetate containing 0.5 mol/L Acetic Acid	14
6	Chitosan	G6000PW + G3000PW	0.5mol/L Sodium Acetate containing 0.5 mol/L Acetic Acid	14
7	Glycol chitosan	G5000PW + G3000PW	0.3mol/L Sodium Sulfate containing 0.5 mol/L Acetic Acid	14
8	Poly(4-vinyl benzyl trimethyl ammonium chloride)	G5000PW + G3000PW	0.1mol/L Sodium Sulfate containing 1~5% Acetic Acid	35
9	Reaction product of cellulose phosphate with N-vinyl-2-pyrrolidone	G4000PW + G3000PW	0.3% Acetic Acid containing 10% CH ₃ CN and 0.1% Triethylamine	15

Table 6 TSK-GEL PW_{XL} Column Performance in the Presence of High Percentage of Selected Organic Solvents

	H ₂ O/MeOH 50/50	H ₂ O/CH ₃ CN 50/50	H ₂ O/HCOOH 50/50	H ₂ O/DMSO 50/50
TSKgel G2500PW _{XL}	1) 15,200	1) 14,700	1) 15,600	1) 16,600
	2) 15,100	2) 15,200	2) 15,300	2) 18,000
	3) 14,800	3) 15,000	3) 14,200	3) 17,400
TSKgel G3000PW _{XL}	1) 17,200	1) 16,000	1) 18,300	1) 18,000
	2) 16,500	2) 16,500	2) 19,100	2) 18,500
	3) 16,200	3) 15,700	3) 18,600	3) 18,700
TSKgel G4000PW _{XL}	1) 13,100	1) 12,900	1) 12,600	1) 13,000
	2) 13,700	2) 12,700	2) 12,800	2) 12,700
	3) 13,300	3) 13,000	3) 12,500	3) 13,200
TSKgel G5000PW _{XL}	1) 12,400	1) 13,000	1) 12,400	1) 13,700
	2) 11,000	2) 12,500	2) 12,000	2) 13,700
	3) 11,800	3) 12,300	3) 11,800	3) 13,900
TSKgel G6000PW _{XL}	1) 7,800	1) 8,800	1) 8,000	1) 8,800
	2) 7,300	2) 8,100	2) 7,800	2) 8,800
	3) 8,200	3) 8,400	3) 7,800	3) 8,200
TSKgel GMPW _{XL}	1) 7,600	1) 7,700	1) 7,200	1) 7,400
	2) 6,900	2) 7,400	2) 8,100	2) 6,600
	3) 7,500	3) 7,800	3) 7,300	3) 7,600
TSKgel G-Oligo-PW	1) 16,200	1) 17,200	1) 16,400	1) 14,800
	2) 17,100	2) 17,400	2) 16,000	2) 15,200
	3) 16,900	3) 16,900	3) 16,100	3) 14,200

Note

- 1) Theoretical plate number measured before testing.
- 2) Theoretical plate number measured after first solvent exchange.
- 3) Theoretical plate number measured after second solvent exchange.

The measurement conditions are the same as in Table 1.

The applicability of higher concentrations of several important solvents was confirmed as shown in Table 6. Changing from one solvent to another was carried out slowly (flow rate at 0.5mL/min) with linear gradient according to the procedure depicted in Figure 13. Typical examples of changes in column pressure drop during the solvent change-over are shown in Figure 14. The data in Table 6 indicates that all columns tested were compatible with 50% aqueous solutions of methanol, acetonitrile,

formic acid, and dimethyl sulfoxide when the solvent exchange was performed as described.

(2) pH

TSK-GEL PW_{XL}-type columns can be used at both high pH and low pH at room temperature. However, the use of alkaline or acidic solutions at high temperatures is not recommended as this can result in column failure.

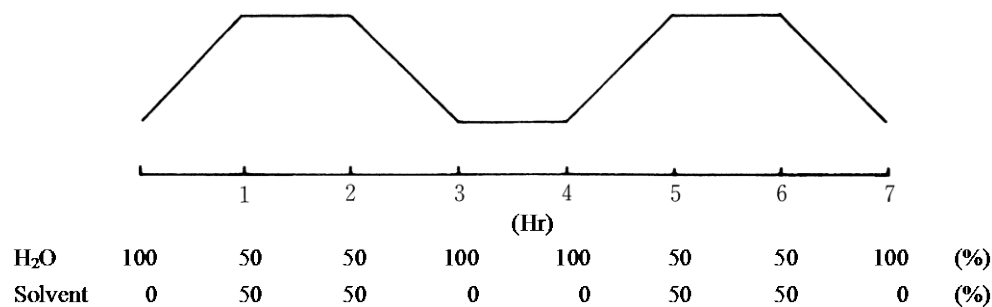


Fig. 13 Solvent Exchange Procedure Used for Experiments in Table 6

Condition: Organic Solvents from Water, 60min. Linear gradient

Flow rate: 0.5mL/min

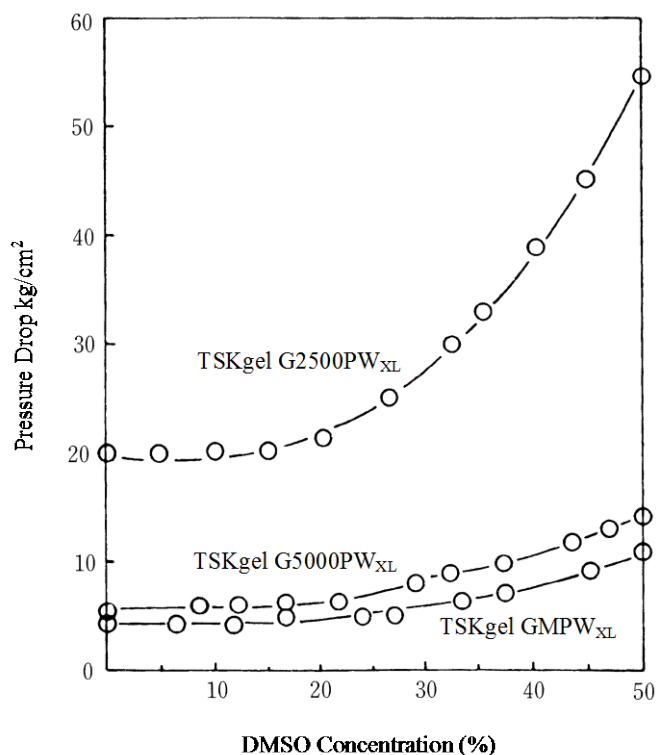


Fig. 14 Relationship between Eluent Composition and Pressure Drop

Column: (1) TSKgel G2500PW_{XL}

(2) TSKgel G5000PW_{XL}

(3) TSKgel GMPW_{XL}

Column size: 7.8mm ID × 30cm

Flow rate: 0.5mL/min

4. Column selection

To make the best use of a high performance GFC column, careful consideration is necessary. Since Tosoh's high performance line of TSK-GEL GFC columns consist of eighteen grades, namely three of TSK-GEL SW-type, seven of the conventional TSK-GEL PW-type and eight of the new TSK-GEL PW_{XL}-type, it is not easy to select the best column for each purpose.

Table 7 provides general guidance to select the appropriate column from the perspective of routine samples analysis. Various factors should be taken into account such as resolving power, molecular weight separation range, linearity of the calibration curve, adsorptive properties and analyte recovery, solvent compatibility, column life time, sample loading capacity, etc.

4-1. Column selection between PW and SW

In general, TSK-GEL SW columns are suitable for the separation of monodisperse biopolymers such as proteins and nucleic acids due to their excellent resolving power. TSK-GEL PW columns are selected for the separation of polydisperse polymers such as polysaccharides and synthetic water-soluble polymers because of their larger exclusion limits and linearity of calibration curves.

Table 7 Column Selection Guide for High Performance GFC

Sample		Column Selection		Selection Criteria
		First selection	Second selection	
Carbohydrates	polysaccharides	TSKgel GMPW _{XL}	TSKgel G5000PW _{XL} + G3000PW _{XL}	large pore size linearity of calibration curve
	oligosaccharides	TSKgel G-Oligo-PW	TSKgel G2500PW _{XL} TSKgel G2000PW	resolving power
Nucleic Acids	DNA fragments	large	TSKgel G-DNA-PW TSKgel G5000PW _{XL}	large pore size resolving power
		medium & small	TSKgel G4000SW TSKgel G3000SW	suitable pore size resolving power
	RNA	TSKgel G4000SW TSKgel G3000SW		
	oligonucleotides	TSKgel G2500PW _{XL}		small pore size ionic interaction
Proteins	normal size proteins	TSKgel G3000SW TSKgel G4000SW TSKgel G2000SW	TSKgel G3000PW _{XL} TSKgel G4000PW _{XL}	resolving power
	large proteins	low density lipoprotein	TSKgel G6000PW _{XL} TSKgel G5000PW _{XL}	large pore size resolving power
		gelatin	TSKgel GMPW _{XL}	TSKgel G5000PW _{XL} + G3000PW _{XL}
Peptides	large	TSKgel G3000SW TSKgel G2000SW	TSKgel G3000PW _{XL}	
	small	TSKgel G2500PW _{XL}	TSKgel G2000SW	linearity of calibration curve resolving power
Virus		TSKgel G6000PW _{XL} TSKgel G5000PW _{XL}		large pore size resolving power
Synthetic polymers		TSKgel GMPW _{XL}	TSKgel G5000PW _{XL} + G3000PW _{XL}	large pore size linearity of calibration curve low adsorption
Synthetic oligomers	nonionic and cationic	TSKgel G-Oligo-PW	TSKgel G2500PW _{XL}	small pore size resolving power ionic interaction
	anionic	TSKgel G2500PW _{XL}		

Polysaccharides

Nonionic polysaccharides are some of the simplest compounds to analyze by GFC because they seldom show non-ideal size exclusion effects on both TSK-GEL PW and SW columns. Since nonionic polysaccharides usually have a wide molecular weight distribution, TSK-GEL PW columns are generally suitable for their measurement. Alsop et al.¹⁶ demonstrated that a series of the TSK-GEL PW columns (TSKgel G5000PW + G3000PW) was very useful for characterization of a clinical dextran sample. Excellent reproducibility and accuracy of the method were confirmed together with long term stability of the columns.

Kato et al.¹⁷ characterized pullulan using a series of TSK-GEL PW columns (TSKgel G5000PW + G3000PW). Takagi et al.¹⁹ fractionated lily amylose using TSK-GEL PW columns (TSKgel G6000PW + G4000PW + G3000PW). Elution from the columns was monitored with a low-angle laser light scattering detector and a precision differential refractometer. They reported that the technique resulted in significant savings of time and sample compared with conventional methods.

Kato et al.¹⁸ determined molecular weight and molecular weight distribution of hydroxypropyl cellulose and hydroxypropylmethyl cellulose used in the film coating of tablets by GFC equipped with a low angle laser light scattering detector.

Elution patterns of several other polysaccharides such as chondroitinsulfate, alginic acid, hyaluronic acid, mannan, starch, and carboxymethyl cellulose are given in reference No. 14.

Nucleic acids

Kato et al.²¹ investigated the effect of operational variables in GFC of DNA fragments and RNAs using TSK-GEL SW columns and TSK-GEL G5000PW columns.

Although small nucleic acids can be analyzed on TSK-GEL SW columns, large ones (DNA fragments of greater than 250,000 bp and RNA fragments of greater than 1,200,000 nucleotide length) are best analyzed on larger pore size TSK-GEL PW columns such as a TSKgel G-DNA-PW or G5000PW_{XL} column. Since nucleic acids are monodisperse molecules, the high resolving power of the new series of TSK-GEL PW_{XL} columns are more effective than the conventional TSK-GEL PW columns. Table 8 shows the best columns for the separation of double-stranded DNA fragments.

Figure 15 shows how flow rate alters HETP for DNA fragments on a two column TSKgel G5000PW system²¹. In Figure 16 the dependence of elution volume on eluent ionic strength obtained on the TSKgel G5000PW two column system²¹ is indicated.

Table 8 Recommended Columns for Separations of Double-stranded DNA Fragments

Base pairs	Recommended Ccolumn
<80	TSKgel G2000SW, G3000SW
80 ~ 160	TSKgel G3000SW
160 ~ 500	TSKgel G4000SW
500 ~ 1000	TSKgel G5000PW _{XL}
1000 ~ 7000	TSKgel G-DNA-PW

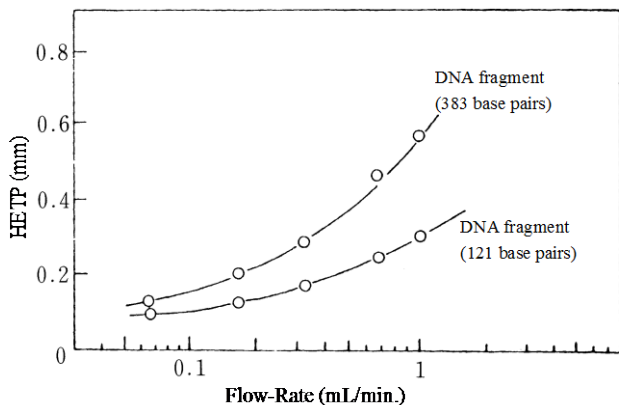


Fig. 15 Dependence of HETP on the Flow Rate for DNA-fragments

Column: TSKgel G5000PW, 7.5mm ID × 60cm × 2
 Eluent: 0.1mol/L Phosphate buffer (pH7.0) + 0.1mol/L sodium chloride and 1mmol/L EDTA

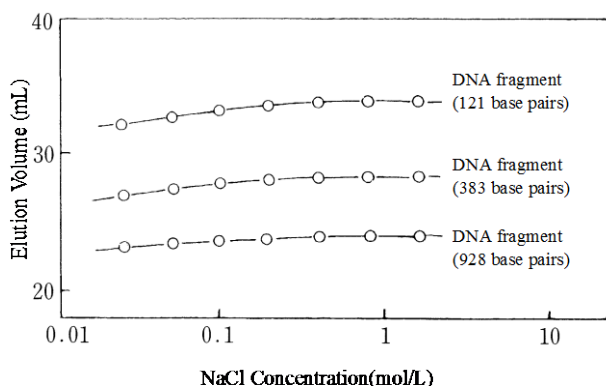


Fig. 16 Dependence of Elution Volume of DNA-fragments on Sodium Chloride Concentration

Column: TSKgel G5000PW, 7.5mm ID × 60cm × 2
 Eluent: 0.01mol/L Tris-HCl buffer (pH7.5) + 0.025 - 1.6mol/L sodium chloride and 1mmol/L EDTA
 Flow rate: 1.0mL/min

Proteins and peptides

The superiority of TSK-GEL SW columns in comparison with TSK-GEL PW columns for the separation of common proteins was pointed out by various authors, including Kato et al.¹¹, Alfredson et al.¹², and Watanabe et al.⁴⁵. The resolving power of size exclusion chromatography depends on the number of theoretical plates, which in turn is determined mainly by the particle size, and on the slope of the calibration curve, which is controlled by such characteristics as pore size, pore size distribution, and pore volume. Although TSK-GEL PW_{XL} columns contain the same particle sizes as TSK-GEL SW columns (or even smaller when compared to TSKgel G4000SW) they are still inferior with regards to the resolution of proteins because the pore distribution is wider and the pore volume is smaller for TSK-GEL PW_{XL} particles. However, it should be pointed out that there are several exceptions in which

TSK-GEL PW_{XL} columns should be the first choice over TSK-GEL SW columns.

They are:

a) When working at high pH (greater than 8), TSK-GEL PW_{XL} columns should be selected rather than TSK-GEL SW columns.

b) Very large proteins such as low density lipoproteins (LDL and VLDL), gelatin, sea worm chlorocruorin, etc., which are excluded even by TSK-GEL G4000SW columns, are better analyzed on TSK-GEL PW_{XL} columns of large pore size such as TSK-GEL G5000PW_{XL} and G6000PW_{XL}.

Hara et al.²²⁻²⁹ investigated the analysis of lipoproteins using TSK-GEL PW columns and TSK-GEL SW columns. Various TSK-GEL PW and SW column systems were investigated. The results are shown in Figure 17.

The most suitable column system to use depends on the application. For the analysis of chylomicron Tosoh recommends a TSKgel G6000PW column. A two column system of TSKgel G5000PW and G3000SW is the best for total pattern analysis. If detailed data of HDL is required, a two column system of TSKgel G3000SW is recommended. Hara et al. also established the analytical methods of lipids contained in lipoproteins such as cholesterol, phospholipid, and triglyceride, using an on-line post-column reaction detector. As lipoproteins are mostly monodisperse polymers, the high performance of the new TSK-GEL PW_{XL} columns is expected to improve this technique to a great extent.

Carrell et al.³⁰ selected a single TSKgel G5000PW column (7.5mm ID × 60cm) for reasons of simplicity, stability, and economy, for analysis and preparative separation of low density lipoprotein. Using a TSKgel G5000PW preparative column, Himmel et al.³¹ found that the pigmented protein, chlorocruorin, isolated from the sea worm *Potamilla leptochaeta*, served as an excellent high molecular weight marker (2.9×10^6) for aqueous size exclusion chromatography. The effect of pH on the elution pattern of gelatin on a two column system of TSKgel G6000PW and G4000PW was reported in a technical report¹⁴ published by Tosoh Corporation.

c) Small peptides are among the most difficult compounds to separate by aqueous size exclusion chromatography. Significant non-ideal size exclusion effects of ionic as well as hydrophobic nature are usually observed both on TSK-GEL PW columns and TSK-GEL SW columns.

Yoshida et al.⁴⁶ gained important insights into this problem using TSK-GEL SW columns with various complex eluents.

Swergold et al.³³ developed a very simple eluent system for the separation of small peptides on a TSKgel G3000PW column. Using an aqueous eluent containing 36-45% acetonitrile and 0.1% trifluoroacetic acid they were able to retain the peptides using a size exclusion mechanism. An advantage of this method is that the eluent is volatile. Tosoh confirmed this technique using a TSKgel G3000PW_{XL} column

as shown in Figure 18 (typical chromatogram) and Figure 19 (calibration curve).

Synthetic water-soluble polymers

For the separation of synthetic water-soluble polymers, TSK-GEL PW-type columns are commonly used due to a much wider separation range, better linearity of calibration curve, and much lower adsorptive properties compared with TSK-GEL SW-type columns.

As indicated by Alfredson et al.¹², TSK-GEL SW-type columns often show high adsorption of linear polymers such as polyvinyl pyrrolidone, polyacrylamide, polyacrylic acid, etc. This may be due to the interaction of residual silanol groups on the surface of TSK-GEL SW-type packings with such polymers. The different elution behavior of these polymers from proteins may be explained as follows: flexible linear polymers can penetrate the chemically bonded organic layer to the point that they interact with silanol groups, while rigid proteins do not.

Carole et al.³⁴ used a two column system consisting of a TSKgel G5000PW and G3000PW column for characterization of poly(vinylalcohol).

Higo et al.³⁵ characterized a cationic polyelectrolyte, poly(4-vinylbenzyl trimethylammonium chloride), using a two column system consisting of a TSKgel G5000PW and G3000PW column. They investigated the effect of eluents, particularly the addition of organic solvents, on the elution pattern and found that normal size exclusion chromatography curves were obtained when 0.1 mol/L sodium sulfate solution containing small amounts of acetic acid were used as an eluent.

Dubin et al.^{36,37} reported that TSKgel G5000PW and G3000PW could successfully be used for measurement of a cationic polymer such as poly(ethylenimine), poly(dimethyldiallylammonium chloride), and polymethacryloxyethyltrimethyl ammonium methosulfate.

Oligomers

In the molecular weight region of less than ca. 3000, TSK-GEL PW-type columns of small pore size such as TSKgel G-Oligo-PW and G2500PW_{XL} are preferred over TSKgel G2000SW because of higher resolving power due to better selectivity and better theoretical plate number.

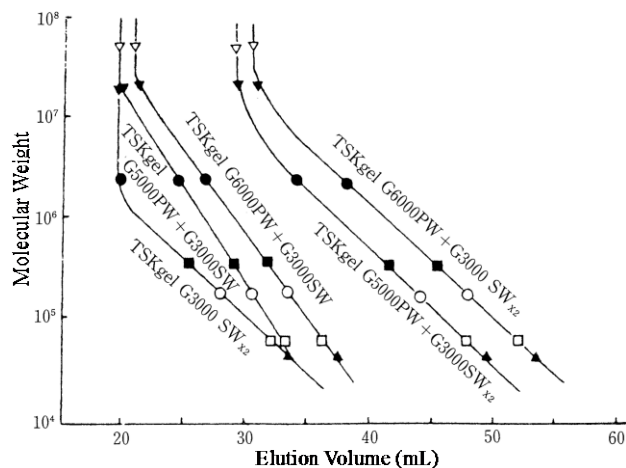


Fig. 17 The Relation between Molecular Weight of Lipoproteins and Elution Volume for the Combination TSK-GEL GFC Columns

Column size: 7.5mm ID × 60cm

Samples: ▽, chylomicron; ▼, VLDL; ●, LDL; ■, HDL₂; ○, HDL₃; □, albumin; ▲, ovalbumin

Eluent: 0.1M Tris-HCl buffer (pH7.4)

Flow rate: 1.0mL/min

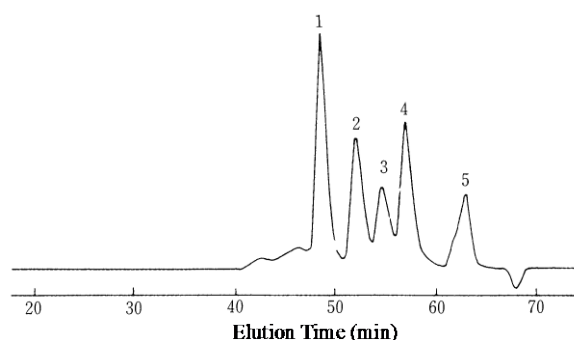


Fig. 18 Elution Pattern of a Peptide Mixture on a TSKgel G3000PW_{XL} column

Column: TSKgel G3000PW_{XL}, 7.8mm ID × 30cm × 2

Sample: peptides

1. aprotinin,
2. insulin β-chain,
3. α-MSH
4. bradykinin potentiator C,
5. glutathione

Eluent: 0.1% TFA/45% CH₃CN

Flow rate: 0.3mL/min

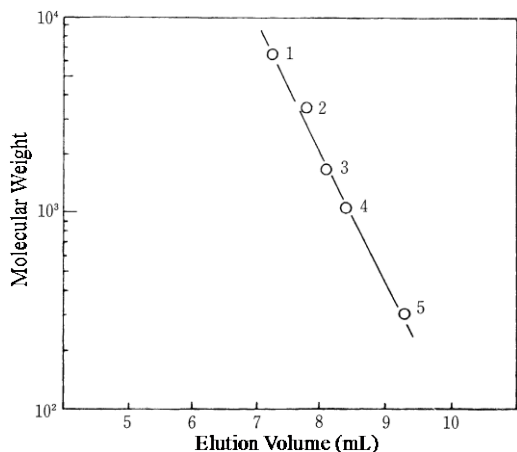


Fig. 19 Peptide Calibration Curves for a TSKgel G3000PW_{XL} column

Column: TSKgel G3000PW_{XL}, 7.8mm ID × 30cm

Sample: 1. aprotinin (6500) 2. insulin β-chain (3400)
3. α-MSH (1665) 4. bradykinin potentiator C (1052)
5. glutathione (307)

Eluent: 0.1% TFA/45% CH₃CN

Flow rate: 1.0mL/min

4-2 Column selection among TSK-GEL PW-type columns

(1) TSK-GEL PW_{XL}- or PW-type columns

For analytical purposes, the new TSK-GEL PW_{XL} columns are recommended given that an HPLC system with sufficiently small dead volume is available.

For preparative separation, particularly when large amounts of sample are to be applied, the TSK-GEL PW columns are recommended because of higher sample loading capacity.

(2) Column selection for the analysis of polydisperse polymers

The availability of mixed-bed linear columns, such as TSK-GEL GMPW_{XL} or GMPW, has simplified column selection for unknown polymer samples. Typically, the analyst first runs the sample on a mixed-bed linear TSKgel GMPW or GMPW_{XL} column. Based on the sample elution profile a column of particular pore size is selected that best covers the whole molecular weight range of the sample. Thus it is recommended to have at least one mixed-bed TSKgel GMPW or GMPW_{XL} column available for the analysis of unknown polymers. Although two column systems, such as TSK-GEL G6000PW and G4000PW, TSK-GEL G6000PW and G3000PW, or TSK-GEL G5000PW and G3000PW, can provide a linear calibration curve over a wide range of molecular weights, using a TSKgel GMPW or GMPW_{XL} column can save time and money compared with those multi-column systems.

(3) Column selection for the analysis of oligomers

For the separation of small oligomers, TSK-GEL G-Oligo-PW or G2500PW_{XL} are the columns of choice. Detailed comparisons of these two columns have been published elsewhere⁴⁷.

A TSKgel G-Oligo-PW column is recommended for the analysis of nonionic and cationic oligomers because of its high resolving power, while a TSKgel G2500PW_{XL} is preferred for anionic oligomers. A specialty line of columns, TSK-GEL PW_{XL}-CP, was introduced in 2007 for cationic polymers. Available grades include TSKgel G3000PW_{XL}-CP, TSKgel G5000PW_{XL}-CP, and TSKgel G6000PW_{XL}-CP

5. Product line of TSK-GEL PW-type columns

Table 9 lists the entire product line of TSK-GEL PW-type columns including both analytical and preparative columns, as well as guard columns.

Table 9 Product Line of TSK-GEL PW- and PW_{XL}-Type Columns

	TSK-GEL PW _{XL} Series		TSK-GEL PW Series					
	7.8mm ID × 30cm		7.5mm ID × 30cm		7.5mm ID × 60cm		21.5mm ID × 60cm	
	Particle size (μm)	TP/column	Particle size (μm)	TP/column	Particle size (μm)	TP/column	Particle size (μm)	TP/column
TSKgel G2500	7	16,000	12	5,000	12	10,000	17	10,000
TSKgel G3000	7	16,000	12	5,000	12	10,000	17	10,000
TSKgel G4000	10	10,000	17	3,000	17	6,000	20	6,000
TSKgel G5000	10	10,000	17	3,000	17	6,000	20	6,000
TSKgel G6000	13	7,000	17	3,000	17	6,000	—	—
TSKgel GMPW	13	7,000	17	3,000	17	6,000	—	—
TSKgel G-Oligo-PW	7	14,000	—	—	—	—	—	—
TSKgel G-DNA-PW	10	10,000	—	—	—	—	—	—

	Column system	Column size
TSKguard Column PW _{XL}	TSKgel G2500PW _{XL} ~ GMPW _{XL}	6.0mm ID × 4cm
TSKguard Column G-Oligo-PW	TSKgel G-Oligo-PW	6.0mm ID × 4cm
TSKguard Column PW	TSKgel G2500PW ~ GMPW	7.5mm ID × 7.5cm
TSKguard Column PW	TSKgel G2500PW ~ G5000PW	21.5mm ID × 7.5cm

Table 10 Range of Elution Conditions for TSK-GEL PW_{XL}-type Columns

Column	Flow Rate		Maximum Pressure drop	Temperature	
	Suitable Range	Maximum		Suitable Range	Maximum
	mL/min	mL/min	kg/cm ²	°C	°C
TSKgel G2500PW _{XL}	0.5-0.8	1.0	40	10-60	80
TSKgel G3000PW _{XL}	0.5-0.8	1.0	40	10-60	80
TSKgel G4000PW _{XL}	0.3-0.6	1.0	20	10-60	80
TSKgel G5000PW _{XL}	0.3-0.6	1.0	20	10-60	80
TSKgel G6000PW _{XL}	0.3-0.6	1.0	20	10-60	80
TSKgel GMPW _{XL}	0.3-0.6	1.0	20	10-60	80
TSKgel G-Oligo-PW	0.5-0.8	1.0	40	10-60	80
TSKgel G-DNA-PW	0.2-0.5	0.6	20	10-60	80

Note that TSK-GEL G1000PW columns are available on our website. Visit www.tosohbioscience.com for details.

6. Some advice for use of TSK-GEL PW_{XL} columns

Each column is shipped with an Operating Conditions and Specification (OCS) sheet that details how to keep the column in good working order, including what to do when the column has become contaminated and/or performance has declined.

Summarized here are several points to consider.

6-1 Range of elution condition

Suitable flow rate range, maximum flow rate, maximum pressure, suitable temperature range and highest temperature are listed in Table 10.

pH range is 2-12 for all TSK-GEL PW_{XL}-type columns.

6-2 Prevention of column deterioration

(1) Filtering of the mobile phase and sample solutions is important to avoid an increase in pressure and decrease of performance due to the clogging of the inlet frit at the top of the gel bed.

(2) The use of a guard column to protect the analytical column is recommended. The guard column should be replaced immediately after any abnormal phenomenon such as an increase in pressure and decrease of performance is observed.

(3) Maintaining the flow rate within the recommended flow rate range rather than at the maximum flow rate will help to avoid bed compression and to prolong column life.

(4) When changing solvents, it is recommended to make gradual changes in solvent composition to protect the bed from compression.

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