TOSOH

SEPARATION REPORT NO. 37

INTRODUCTION OF AQUEOUS SEC COLUMNS: TSKgel PWXL SERIES

Table of Contents

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1. Introduction

During the past decade, high performance gel filtration chromatography=HPGFC (often referred to as aqueous size exclusion chromatography or aqueous gel permeation chromatography) has made remarkable progress. Several excellent reviews[1-7] have been published.

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

Now a new series of TSK-GEL PW Type, consisting of six TSKgel PW_{XL} columns and two special columns (TSKgel G-Oligo-PW and TSKgel G-DNA-PW), has been introduced into the market in order to improve resolution drastically and to cut time required for measurement to a great extent. Besides, some new grades are added to enlarge application range. Main features and improved points of the new series are summarized in comparison with the conventional series as follows :

(1) Higher performance

The numbers of theoretical plate (per unit column length) of the new PW_{XL} series are practically more than double of those of the conventional series. Therefore the resolving power of the new PW_{XL} series is increased around 1.4 times against the conventional series of the same column length. Compared with the conventional series of long columns (60cm), the new PW_{XL} series can reduce measurement time to one half to give nearly equal resolution.

(2) Introduction of TSKgel $GMPW_{XL}$

TSKgel $GMPW_{XL}$ is a new grade featured by excellent linearity of the calibration curve over a very wide range of molecular weight from 5×10^2 to more than 10^7.

(3) Introduction of TSKgel $G2500PW_{XL}$

One of the problems of the current PW Type is that there is a difference in chemical nature between the grade of small pore size (G1000PW and G2000PW) and those of large pore size (G3000PW~G6000PW). The former has a considerable amount of ionic groups (both cationic and anionic), while the latter has only a small amount of weakly anionic groups. Therefore it is not recommendable to use a column of TSKgel G2000PW or TSKgel G1000PW in conjunction with other grades. To improve this situation, TSKgel G2500PW is introduced in both the conventional series and the new PW_{XL} series.

TSkgel G2500PW has almost the same chemical nature as the grades of large pore size and it can be used in conjunction with them. TSKgel G2500PW has almost the same calibration curve as TSKgel G2000PW, but it should be noted that the former is to some extent inferior to the latter in the separation of small molecules.

(4) Introduction of TSKgel G-Oligo-PW

In order to improve the resolution for oligomers further, TSKgel G-Oligo-PW is indroduced as a special grade dedicated to the separation of non-ionic and cationic oligomers such as oligosaccharide, polyethylene glycol etc. The packing of the G-Oligo-PW carries cationic groups just as that of the G2000PW. Therefore the G-Oligo-PW column is not recommended to apply to anionic samples.

(5) Introduction of TSKgel G-DNA-PW

TSKgel G-DNA-PW is a new column specially dedicated to the separation of large polynucleotides (for example, DNA fragments of 500~5000 base pair). TSKgel G-DNA-PW featured by very large pore size (ca. 4000A) and small particle size (10μm) can separate large DNA fragment salmost completely by the difference of half size within 2 ~ 4 hours.

In this paper only fundamental characteristics and properties of the new series will be described together with brief review for column selection. The following matters will be published in detail in near future :

(1) separation of water-soluble oligomers on new TSK-GEL PW columns, (2) separation of water-soluble polymers on new TSK-GEL PW columns, (3) separation of large DNA fragments on TSKgel G-DNA-PW column.

2. Characteristics of PW_{XL} columns

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

Table 2 shows the separation ranges of the series for the PEG and PEO standards.

All of them employ the same column dimmension of 7.8 mm inner diameter and 30 cm length.

As they employ smaller particles, the guaranteed numbers of theoretical plates per unit length are more than 2.8 times compared with those of the corresponding conventional TSKgel PW columns as shown in Table 3.

Table 1 **Characteristics of New Series of TSKgel PW Columns**

Column	ParticleSize μm	Number of Theor. Plate Guaranteed.[*1] (TP/Column)	Exclusion Limit[*2]			Column Dimmension mm ID × cm L
			PEO	Dextran	Protein	
TSKgel G2500PW$_{XL}$	6	14,000	5×10^3			7.8×30
TSKgel G3000PW$_{XL}$	6	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	6	14,000	5×10^3			7.8×30
TSKgel G-DNA-PW	10	10,000	2×10^7			

Note: [*1]Measurement condition for theoretical plate number.
[*2]The values in parentheses are presumed.
Eluent: Distilled water
Flow rate: 1.0mL/min
Sample: Ethylene glycol 1%×20μL

Table 2 **Separation Range of New Series of TSKgel PW Type for PEG and PEO Standard**

Column	Separation Range		
TSKgel G2500PW$_{XL}$		-	3,000
TSKgel G3000PW$_{XL}$		-	40,000
TSKgel G4000PW$_{XL}$	2,000	-	200,000
TSKgel G5000PW$_{XL}$	8,000	-	800,000
TSKgel G6000PW$_{XL}$	40,000	-	8,000,000
TSKgel GMPW$_{XL}$	500	-	8,000,000
TSKgel G-Oligo-PW		-	3,000
TSKgel G-DNA-PW	40,000	-	8,000,000

Table 3 **Comparison of Theoretical Plate Number Guaranteed between New and Old PW Series**

Grade	Old Series		New Series
	7.5 mm ID × 6 cm L	**7.5 mm ID × 3 cm L**	**7.8 mm ID × 3 cm L**
TSKgel G2500PW	10,000 TP/column	5,000 TP/column	14,000 TP/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 1~3 show the calibration curves for TSKgel PW_{XL} columns measured with the above-mentioned standards, respectively.
Figure 4 shows the calibration curve for a TSKgel G-Oligo-PW column (solid line) together with the one for TSKgel G2500 column (dotted line) measured with polyethylene glycol standards. The calibration curve of TSKgel G-DNA-PW for double-stranded DNA fragments will be presented elsewhere[42].

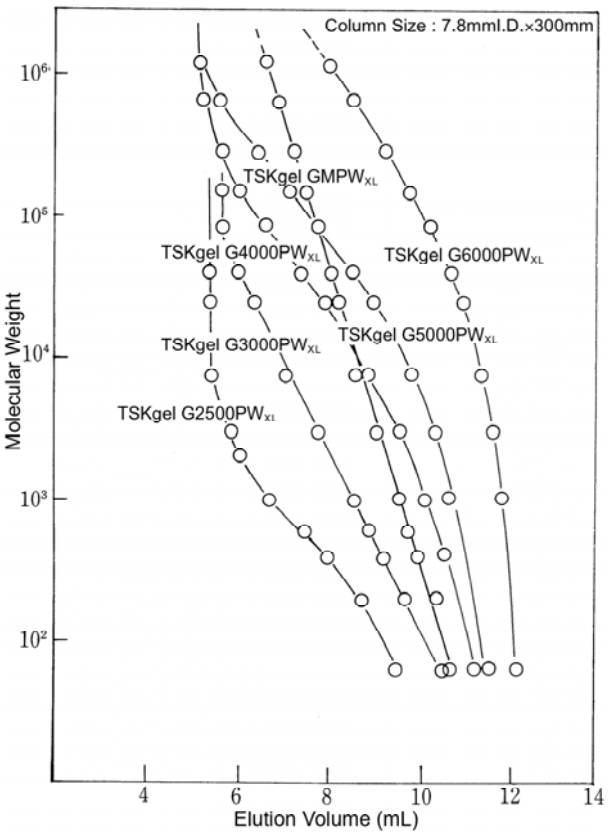

Fig. 1 Calibration Curves of TSKgel PWXL Columns for PEG and PEO Standards
Sample: PEG and PEO Standards
Eluent: Distilled water
Flow rate: 1.0 mL/min.

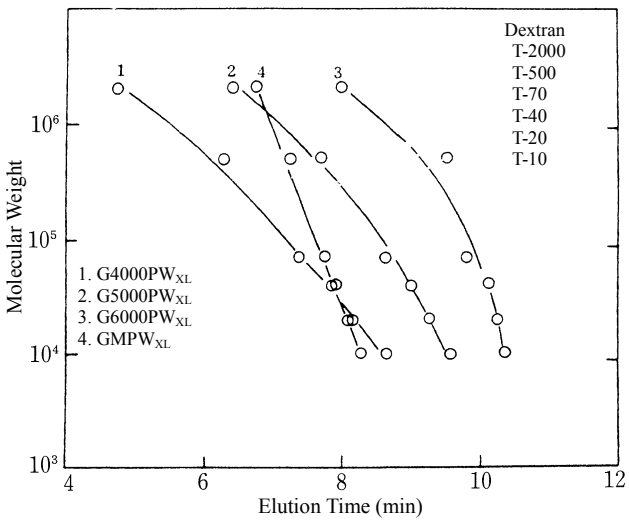

Fig. 2 Calibration Curves of TSKgel PW_{XL} Columns for Dextran Standards
Eluent: 0.2 M P. B. (pH 6.8)
Flow rate: 1.0 mL/min.

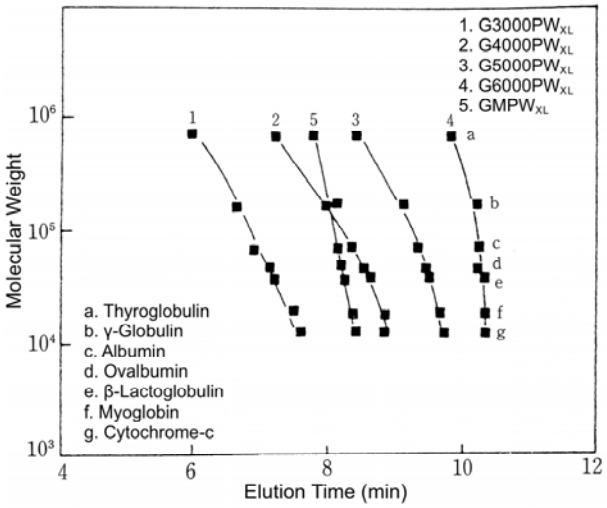

Fig. 3 Calibration Curves of TSKgel PW_{XL} Columns for Proteins
Eluent: 0.2 M P. B. (pH 6.8)
Flow rate: 1.0 mL/min.

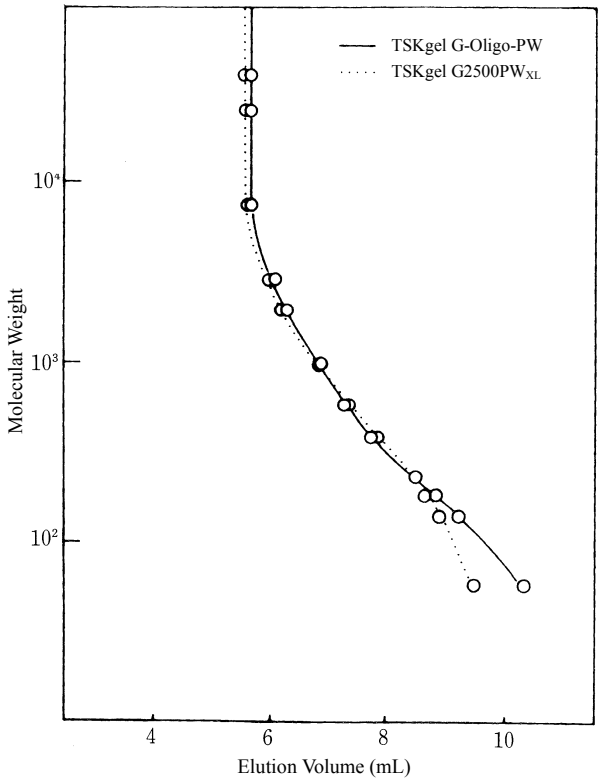

Fig. 4 Calibration Curves of TSKgel G-Oligo-PW and TSKgel G2500PW$_{XL}$
Column size: 7.8 mm ID × 30 cm L
Sample: PEG and PEO Standards
Eluent: Distilled water
Flow rate: 1.0 mL/min.

3. Basic properties of 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 of an eluent etc. As a typical example, Fig. 5 shows the flow rate dependence of the number of theoretical plates measured with ethylenglycol (a typical small molecule) on a TSKgel G2500PW_{XL} column (employing the smallest particle size 6μm among PW_{XL} series), and that measured with a PEO standard (a typical large molecule) on a TSKgel G6000PW_{XL} column (employing the largest particle size 13μm among PW_{XL} series). The number of theoretical plates for the former is almost constant, while that for the latter decreases considerably as flow rate increases. Thus it is recommended to use lower flow rate for the grades of large pore size which are used for large molecules.

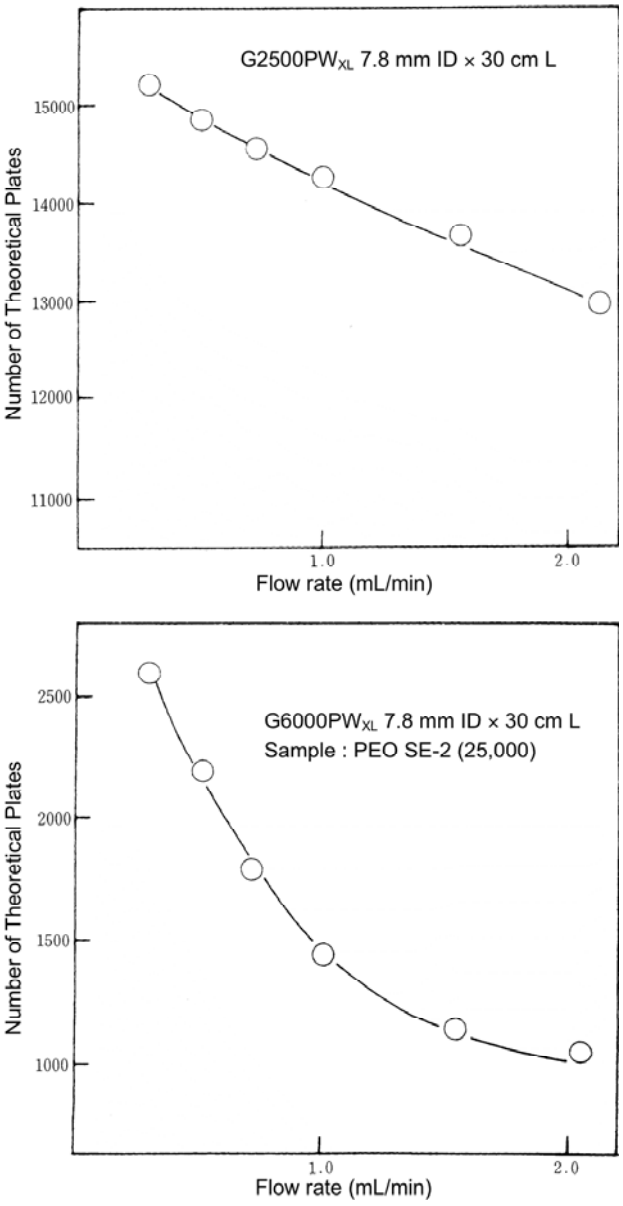

Fig. 5 Flow Rate Dependence of the Number of Theoretical Plates on TSKgel G2500PWXL and G6000PWXL

3-2. Ionic properties

Figure 6 shows the curves of the PW_{XL} gels titrated with 0.1 N sodium hydroxide. All of them have small amount of weakly anionic groups. At low ionic strength of an eluent anionic samples are excluded by ionic repulsion to elute earlier than theoretically expected, while cationic samples are retarded by ionic adsorption to elute later than theoretically expected. In order to eliminate such ionic interactions, it is common to use an eluent with ionic strength of more than 0.1μm.

Figure 7 displayes the difference of the titration curves between G2000PW and G2500PW packings. It is clear that the latter is much improved in the ionic property. The titration curve of the packing of G-Oligo-PW is almost the same as that of G2000PW.

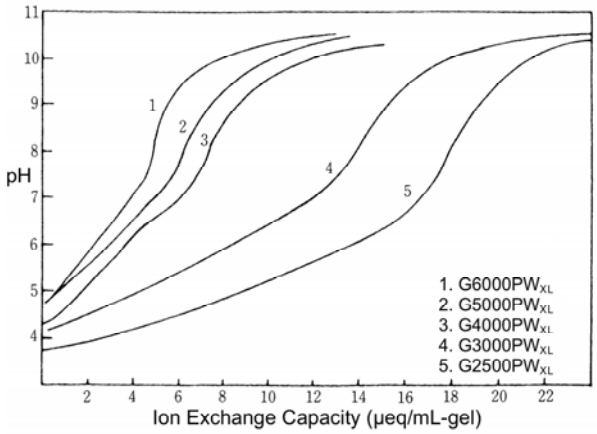

Fig. 6 Titration Curves of PW_{XL} Gels

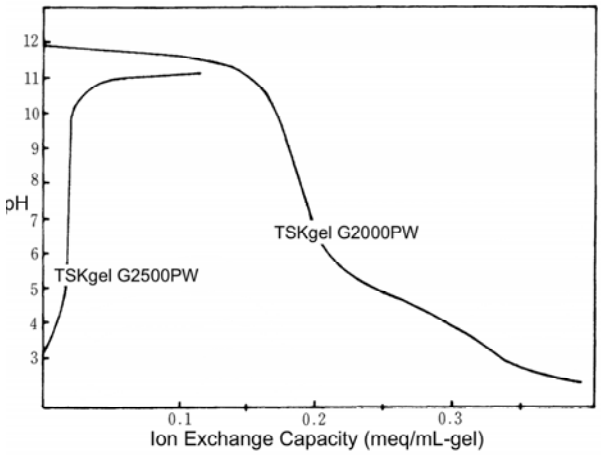

Fig. 7 Comparison of Titration Curves between G2500PW and G2000PW Gels

Figure 8 shows the effect of sodium chloride concentration on the elution volume of adenosine monophosphate (a typical anionic sample) on a G2500PW$_{XL}$ and G-Oligo-PW column. It can be seen that the latter shows strong interaction as the NaCl concentration decreases.

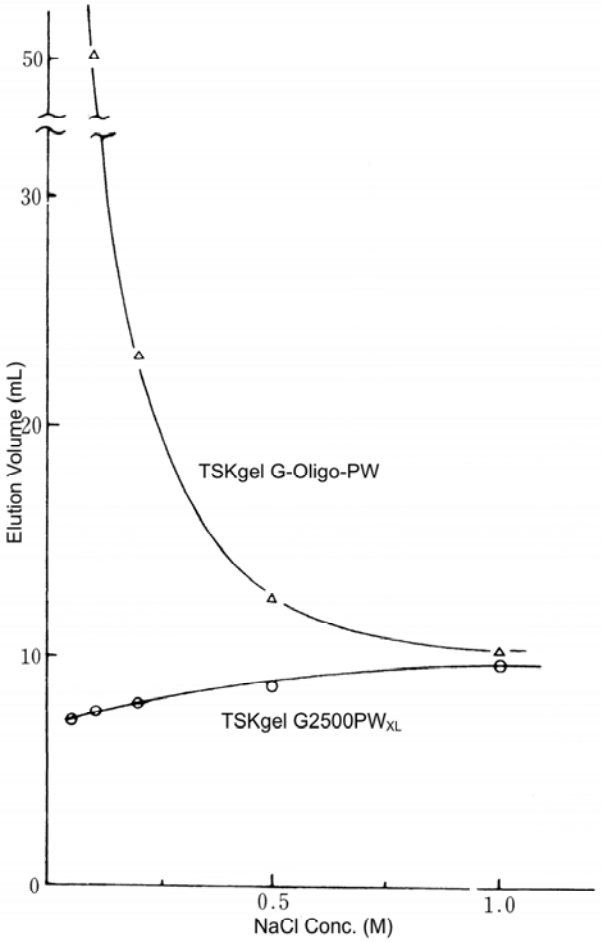

Fig. 8 Dependence of Elution Volume of Adenosine Monophosphate on Salt Concentration

Sample: Adenosine monophosphate
Column size: 7.8 mm ID × 30cm L
Eluent: 0.02M P. B. (pH6.8) + 0.05M-1.0M NaCl

3-3. Hydrophobic property

The PW gels show higher hydrophobicity than polysaccharide gels such as crossliked dextran gels. In Table 4 capacity factors of several alcohols on TSKgel G2500PW$_{XL}$ are shown. The longer the alkyl group, the larger the retardation becomes. The hydrophobic interaction tends to be stronger as salt concentration of an eluent increases, while it can be reduced by addition of an organic solvent into the eluent. The dependence of elution volume of alcohols on sodium chloride concentration is shown in Figure 9. Figure 10 shows the dependence of elution volumes of β-phenethyl alcohol, adenine, adenosine and tryptophan on acetonitrile concentration. The samples used in this experiment are typical water-soluble small compounds which show strong interaction with PW gels. As clearly seen from Figure 10, they elute at almost normal position at 50% acetonitrile concentration.

The hydrophobic interaction can also be reduced at high temperatures as shown in Figure 11 which gives the capacity factor dependence of β-phenethyl alcohol on temperature. The effect of acetonitrile concentration (0, 10, and 30%) is also given.

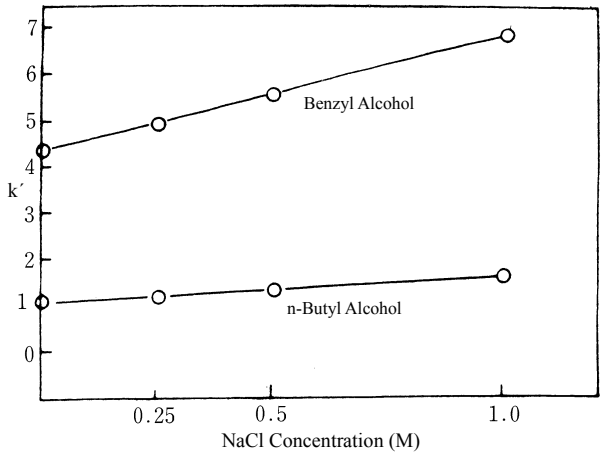

Fig. 9 Dependence of Capacity Factor of Benzyl Alcohol and n-Butyl Alcohol on Sodium Chloride Concentration

Column: TSKgel G2500PW$_{XL}$
Column size: 7.8 mm ID × 30cm L
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	η-Butyl Alcohol	β-Phenethyl Alcohol
G2500PW$_{XL}$	0.16	0.45	0.93	5.53
G3000PW$_{XL}$	0.14	0.35	0.82	5.20
G4000PW$_{XL}$	0.09	0.22	0.49	2.84
G5000PW$_{XL}$	0.07	0.19	0.44	2.84
G6000PW$_{XL}$	0.05	0.15	0.37	2.55

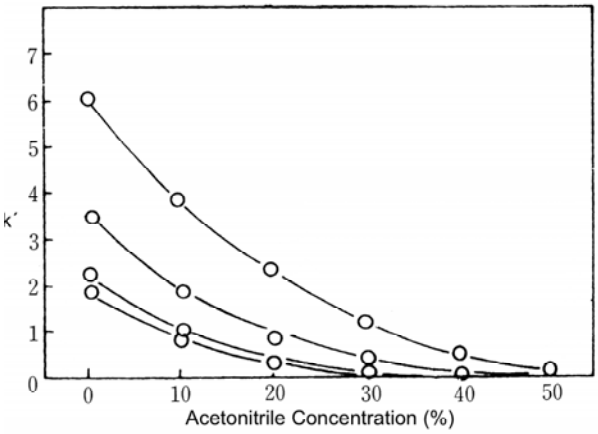

Fig. 10 Dependence of Capacity Factors of β-Phenethyl Alcohol, Adenine, Adenosine and Tryptophan on Acetonitrile Concentration

Column: TSKgel G2500PW$_{XL}$
Column size: 7.8 mm ID × 30 cm L
Sample: (a) β-Phenethyl alcohol
(b) Adenine
(c) Adenosine
(d) Tryptophan
Flow rate: 1.0mL/min.

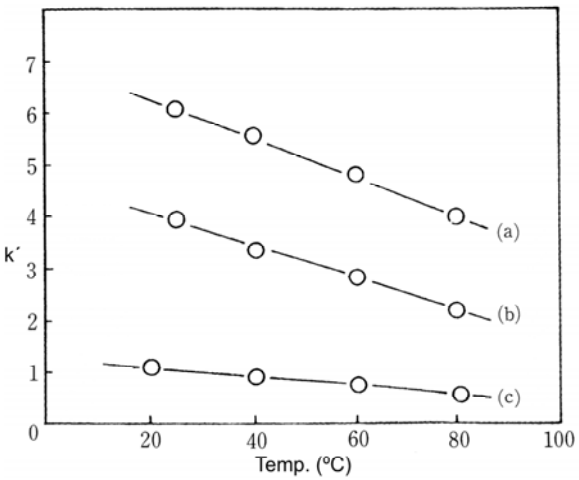

Fig. 11 Dependence of Capacity Factors of β-Phenethyl Alcohol on Temperature

Column: TSKgel G2500PWXL
Column size: 7.8 mm ID × 30 cm L
Sample: β-Phenethyl alcohol
Eluent: (a) Water
(b) Acetonitrile 10% Soln.
(c) Acetonitrile 30% Soln.
Flow rate : 1.0 mL/min.

3-4. Temperature stability

PW gels themselves are thermally so stable in neutral aqueous solutions as to be autogroved at 120□. Columns can be used below 80□ with common neutral aqueous solutions. The solutions of high or low pH should not be used at high temperatures.

Figure 12 shows an example of a running life test of the columns of TSKgel GMPW$_{XL}$, TSKgel G2500PW$_{XL}$ and TSKgel G-Oligo-PW at 60□. During the continuous testing of three months, the numbers of theoretical plates and the pressure drops were kept almost constant.

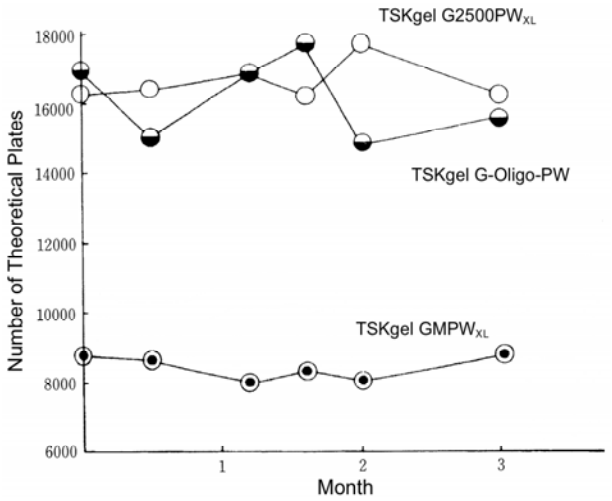

Fig. 12 An Example of Column Life Test at 60ºC

Column size: 7.8 mm ID × 30 cm L
Sample: Ethylene glycol (Condition)
Running Condition;
Flow rate: 1.2 mL/min.
Temp.: 60 ºC
Sample: Ethylene glycol
Measuring Condition;
Flow rate: 1.0 mL/min.
Temp.: 25 ºC

3-5. Solvent compatibility

(1) Organic solvent

Water-soluble organic solvents are frequently used as a modifier in order to suppress hydrophobic interaction between PW columns and samples. Typical examples are listed in Table 5.

All PW columns including the new series except G-DNA-PW are compatible with at least 20 percent aqueous solutions of 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	Column	Eluent	Reference
1	Peptide	G3000PW	0.1% TFA Containing 36-45% CH_3CN	32, 33
2	Poly (vinyl pyrrolidone)	G5000PW + G3000PW	0.1M Sodium Acetate Containing 20% CH_3CN	14
3	Poly (styrene sulfonate)	G6000PW + G3000PW	0.2M Phosphate Buffer Containing 10% CH_3CN	14
4	Poly (dimethyl aminoethyl methacrylate)	G6000PW + G3000PW	0.5M Sodium Acetate Containing 0.5M Acetic Acid	14
5	Poly (ethyleneimine)	G6000PW + G3000PW	0.5M Sodium Acetate Containing 0.5M Acetic Acid	14
6	Chitosan	G6000PW + G3000PW	0.5M Sodium Acetate Containing 0.5M Acetic Acid	14
7	Glycol chitosan	G5000PW + G3000PW	0.3M Sodium Sulfate Containing 0.5M Acetic Acid	14
8	Poly (4-vinyl benzyl trimethyl ammonium chloride)	G5000PW + G3000PW	0.1M 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_3CN and 0.1% Triethylamine	15

Table 6 Applicability of High Concentration of Some Organic Solvents

	H_2O/MeOH 50/50		H_2O/CH_3CN 50/50		H_2O/HCOOH 50/50		H_2O/DMSO 50/50	
$G2500PW_{XL}$	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
$G3000PW_{XL}$	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
$G4000PW_{XL}$	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
$G5000PW_{XL}$	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
$G6000PW_{XL}$	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
$GMPW_{XL}$	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
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 condition is the same as that in Table 1.

The applicability of higher concentrations of several important solvents was confirmed as shown in Table 6. Solvent exchange operation was carried out slowly (flow rate at 0.5mL/min) with linear gradient according to the procedure in Figure 13. Typical examples of the change of the pressure drops during the solvent exchange is shown in Figure 14. It can be seen that all columns tested are compatible with 50 percent aqueous solutions of methanol, acetonitrile, formic acid and dimethyl sulfoxide, if the solvent exchange is performed carefully.

(2) pH

PW_{XL} columns can be used at both high pH1[2)] and low pH[2)] at room temperature.

The use of alkaline or acidic solutions at high temperatures is prohibited because packings will be damaged.

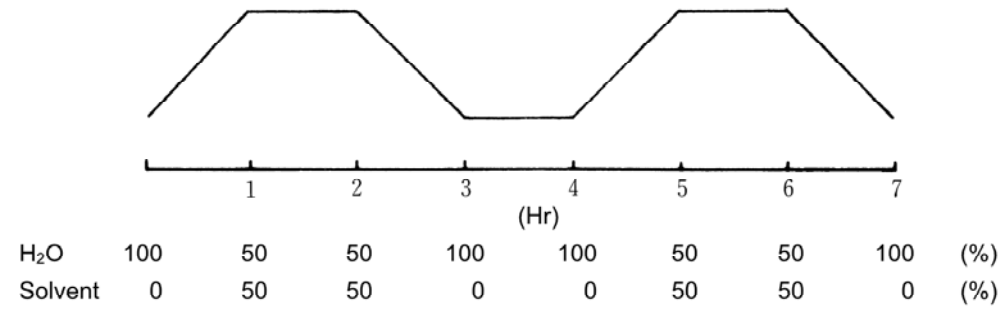

Fig. 13 Solvent Exchange Procedure Used for Experiments in Table 6

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

Flow rate: 0.5 mL/min.

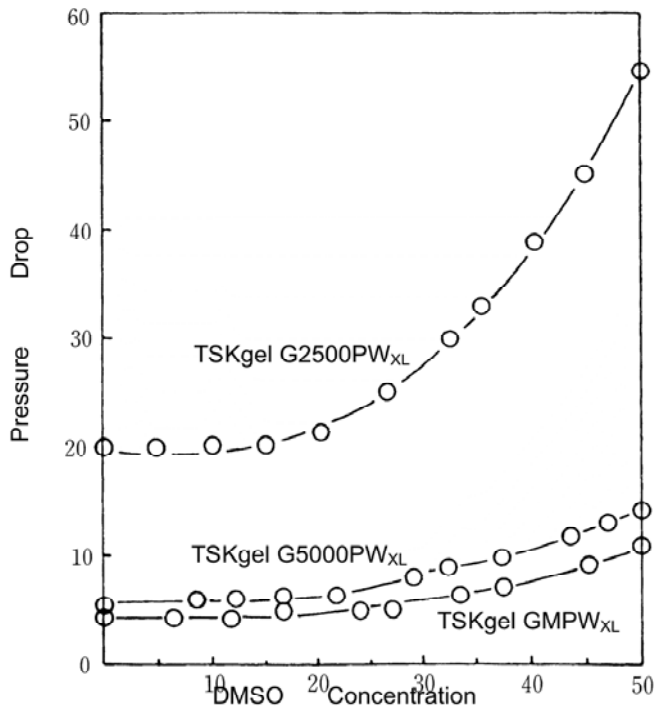

Fig. 14 Relation between Eluent Composition and Pressure Drop

Column: (1) TSKgel G250OPW$_{XL}$
(2) TSKgel G5000PW$_{XL}$
(3) TSKgel GMPW$_{XL}$

Column size: 7.8mml. D.× 30cm

Flow rate: 0.5mL/min.

4. Column selection

To make the best use of the HP GFC column, careful selection is necessary. Since HP GFC series of TSK columns consist of totally eighteen grades, namely three of TSKgel SW Type, seven of the conventional TSKgel PW Type and eight of the new series of TSKgel PW Type, it is not easy to select the best column for each purpose.

In Table 7 a rough idea for the column selection from the view point of analytical use is summarized according to typical samples. Various factors should be taken into consideration such as resolving power, separation range of molecular weight, linearity of calibration curve, adsorptive properties and recovery of sample, solvent compatibility, life time, sample loading capacity, systems at hand, etc.

4-1. Column selection between PW and SW

It can be generally said that SW columns are suitable for the separation of monodisperse biopolymers such as proteins and nucleic acids due to higher resolving power. while PW column are chozen for the separation of polydisperse polymers such as polysaccharides and synthetic water-soluble polymers due to larger exclusion limits and linearity of calibration curves.

(1) Polysaccharides

Nonionic polysaccharides are one of the most simple compounds for GFC because they seldom show nonsize exclusion effects to both PW and SW columns. Since they usually have wide molecular weight distribution, PW columns are generally suitable for their measurement. Alsop et al[16] demonstrated that a series of the PW columns (G5000PW+G3000PW) was very useful for

Table 7 Column Selection Guide for High Performance GFC

Sample			Column Selection		Point in selection
			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$_{XL}$	large pore size linearity of calibration curve
Peptides	**large**		TSKgel G3000SW TSKgel G2000SW	TSKgel G3000PW$_{XL}$	
	small		TSKgel G25000PW$_{XL}$	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$_{XL}$		

characterization of clinical dextran. Excellent reproducibility and accuracy of the method were confirmed together with long term stability of the columns over two years.

Kato et al[17] characterized pullulan using a series of PW columns (G5000PW + G3000PW). Takagi et al[19] fractionated lily amylose using PW columns (G6000PW + G4000PW + G3000PW). Elution from the columns was monitored with a low-angle laser light scattering photometor and a precision differential refractometer. They reported that the technique saved time and sample significantly compared with the conventional methods. Kato et al[18] measured molecular weight and molecular weight distribution of hydroxypropyl cellulose and hydroxypropylmethyl cellulose used in the film coating of tablets by HPGFC equipped with a low angle laser light scattering photometer. They used four column systems of the conventional PW columns.

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

(2) Nucleic acids

Kato et al[21] investigated the effect of operational variables in HPGFC of DNA fragments and RNAs using TSKgel SW columns and TSKgel G5000PW columns.

Although small nucleic acids can be covered by SW columns, large ones (more than 250,000 of double-stranded DNA fragments and more than 1,200,000 of RNAs) should be covered by the PW columns of large pore size such as G-DNA-PW and G5000PW$_{XL}$. Since nucleic acids usually exist as a monodisperse molecule, the high resolving power of the new series of PW columns are quite effective compared with the conventional PW columns. Table 8 shows the best columns for the separation of double-stranded DNA fragments.

Figure 15 shows the flow rate dependence of HETP for DNA fragments on the G5000PW two-column system[21]. Figure 16 shows dependence of elution volume on eluent ionic strength obtained on the G5000PW two-column system[21].

Table 8 Best Column for Separations of Double-stranded DNA Fragments

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

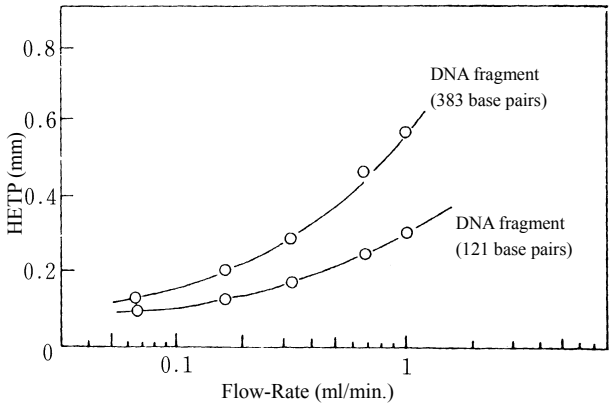

Fig. 15 Dependence of HETP on the Flow Rate for DNA-fragments

Column: TSKgel G5000PW
Column size: 7.5 mmI. D. × 60cm × 2
Eluent: 0.1M phosphate buffer (pH7.0) + 0.1M Sodium chloride and 1mM EDTA

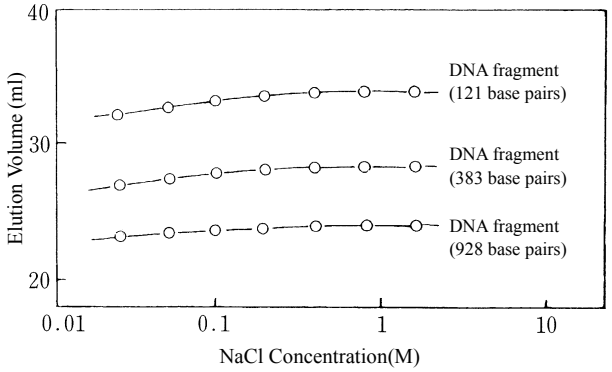

Fig. 16 Dependence of Elution Volume of DNA-fragments on Sodium Chloride Concentration

Column: TSKgel G5000PW
Column size: 7.5 mmI.D. × 60cm × 2
Eluent: 0.01M Tris-HCl buffer (pH7.5) + 0.025 –- 1.6M Sodium chloride and 1mM EDTA
Flow rate: 1.0mL/min.

(3) Proteins and peptides

The superiority of SW columns in comparison with PW columns for the separation of common proteins was described by various authors such as Kato et al[11], Alfredson et al[12]) and Watanabe et al[46]. The resolving power of size exclusion chromatography mainly depends upon the theoretical plate number determined by mainly particle size of packings and the slope of calibration curve determined by pore characteristics such as pore size, pore size distribution and pore volume. Although PW$_{XL}$ columns employ the same particle sizes as SW columns (or even smaller in comparison with G4000SW) they are still inferior in New Series of TSKgel PW Type for High

Performance Gel Filtration Chromatography the resolving power for proteins because of wider pore distribution and smaller pore volume. However it should be noted that there are several exceptions in which PW columns should be the first choice against SW columns as follows;

a) When the use of a high pH solution (higher than 8) can not be avoided, PW columns should be selected.

b) Very large proteins such as low density lipoproteins (LDL and VLDL), gelatin, sea worm chlorocruorin etc. which are excluded even by G4000SW column can be covered by PW columns of large pore size such as G5000PW_{XL}, G6000PW_{XL} etc.

Hara et al[22-29] investigated the analytical method of lipoproteins using PW columns and SW columns in detail. Various column systems in conjunction of large pore size PW columns with SW columns were examined as shown in Figure 17.

The most suitable column system depends on the purpose of analysis. If information of chylomicron, the largest component, is necessary, TSkgel G6000PW is recommended. A two column system of a G5000PW and G3000SW is the best for total pattern analysis. If detailed information of HDL is required, a two column system of TSKgel G3000SW is preferred. Hara et al also established the analytical methods of lipids contained in lipoproteins such as cholesterol, phospholipid and triglyceride using on-line postreaction procedures. Since lipoproteins are obviously monodisperse polymers, the high performance of the new PW columns is expected to improve this technique to a great extent.

Carrell et al[30] selected a single G5000PW column (7.5mmI. D. $\times$ 60cm) due to its relative simplicity, stability, and economy in their work of analytical and preparative separation of low density lipoprotein. By the use of a 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-mohecular-weigh marker (2.9×10^6) for aqueous size exclusion chromatography. The effect of pH on the elution patten of gelatin on a two column system of G6000PW and G4000PW was reported in the technical report[14] published by Toyo Soda.

c) Small peptides

Small peptides are one of the most difficult compounds to be covered by aqueous size exclusion chromatography. Complex, strong, nonsize exclusion effects, both ionic and hydrophobic, are usually observed both on PW columns and SW columns.

Yoshida et al[47] struggled to solve this problem using SW columns with various complex eluents, resulting in making the difficulty of this matter clearer.

Swergold et al[33] developed a very simple eluent system for the separation of small peptides on a TSKgel G3000PW. The system consisting of 36~45 percent acetonitrile solution and 0.1 percent trifluoroacetic acid worked very well according to the size exclusion mechanism. This technique is also featured by the volatility of the eluent. We have confirmed that this technique is covered by a new TSKgel G3000PW_{XL} column as shown in Figure 18 (typical chromatogram) and Figure 19 (calibration curve).

(4) Synthetic water-soluble polymers

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

As indicated by T. Alfredson et al[12], SW columns often show high adsorption to 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 the packings with such polymers. The different elution behavior of these polymers from proteins may be explained as follows: flexible linear polymers can penetrate so deep into the chemically bonded organic layer to interact with silanol groups, while rigid proteins can not.

New Series of TSK-GEL PW Type for High Performance Gel Filtration Chromatography

Carole et al[34] used a two column system consisting of a 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 G5000PW and G3000PW column. They investigated the effect of eluents, particularly addition of organic solvents, on the elution pattern and found that normal size exclusion chromatography curves were obtained when 0.1M 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 cationic polymer such as poly (ethylenimine), poly (dimethyldiallylammonium chloride) and polymethacryloxyethyltrimethyl ammonium methosulfate.

(5) Oligomers

In the molecular weight region of less than ca. 3000, PW columns of small pore size such as G-Oligo-PW and G2500PW$_{XL}$ are recommended against G2000SW because of higher recolving power due to better selectivity and better theoretical plate number.

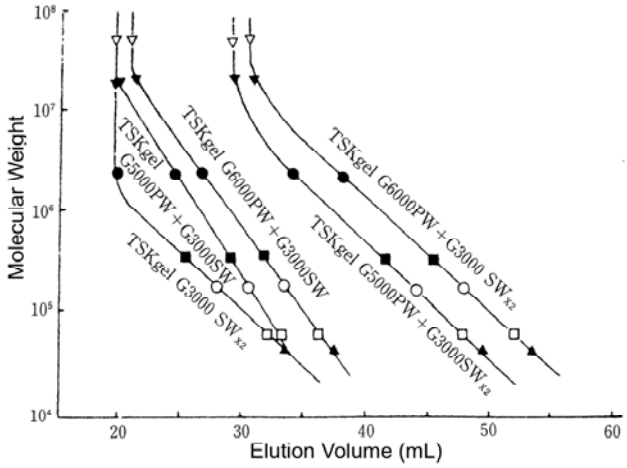

Fig. 17 The Relation between Molecular Weight of Lipoproteins and Elution Volume for the Combination GFC Columns

Column size: 7.5mmI. D. × 60cm
Sample: □ chylomicron; ▼ VLDL; ● LDL; ■ HDL_2 ○ HDL_3; □ albumin; ▲ ovalbumin
Eluent: 0.1M Tris-HCl buffer(pH7.4)
Flow rate: 1.0mL/min.

4-2 Column selection among PW columns

(1) New PW$_{XL}$ columns or Old PW columns

For the analytical purposes, new PW$_{XL}$ columns are recommended, if an up-to-date system with sufficiently small dead volume is available. It should be noted that large dead volume of a HPLC system will kill the high performance of the PW$_{XL}$ columns.

For preparative separation, particularly when large amount mples should be applied, the old PW columns are recommended because of larger sample loading capacity.

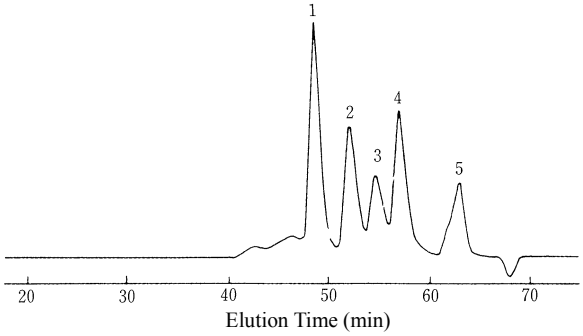

Fig. 18 Elution Pattern of a Peptide Mixture on TSKgel G3000PWXL

Column: TSKgel G3000PW$_{XL}$
Column size: 7.8mmI. D. × 30cm × 2
Sample: peptides
1=aprotinin,
2=insulin B-chain,
3=α-MSH
4=bradykinin potentiator C,
5=glutathione
Eluent: 0.1% TFA/45% CH_3CN
Flow rate: 0.3mL/min.

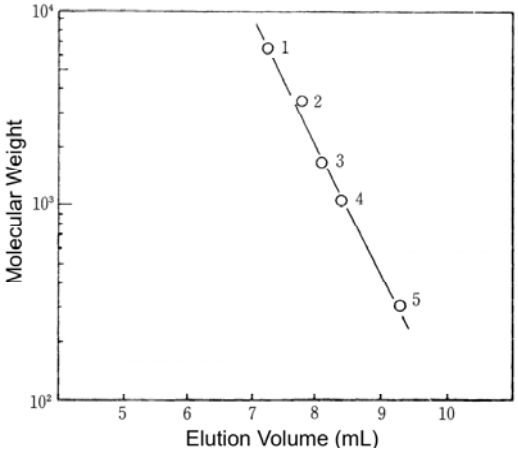

Fig. 19 Peptide Calibration Curves for TSKgel G3000PW$_{XL}$

Column: TSKgel G3000PWXL
Column size: 7.8mmI. D. × 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% CH_3CN
Flow rate: 1.0mL/min.

(2) Selection in the separation of polydisperse polymers

The introduction of $GMPW_{XL}$ or GMPW has made this problem easier. It is a typical procedure for the selection of the best column to test a polymer sample with a GMPW or $GMPW_{XL}$ column at first. Then the best column should be selected to cover the whole molecular weight of the sample and to use the effective separation range as wide as possible. Thus it is recommended to have at least one column of GMPW or $GMPW_{XL}$ in the separation of polymers. So far two column systems such as G6000PW and G4000PW, G6000PW and G3000PW, and G5000PW and G3000PW have played an important role in supplying a linear calibration curve over wide range of molecular weight. Use of a GMPW or $GMPW_{XL}$ column can save time and economy compared with those multi-column systems.

(3) Selection in the separation of oligomers

In the separation of small oligomers, G-Oligo-PW or $G2500PW_{XL}$ is the best. Detailed comparison of these two columns will be published elsewhere[48].

G-Oligo-PW is recommended for nonionic and cationic oligomers because of higher resolving power, while $G2500PW_{XL}$ is recommended for anionic oligomers because of better ionic property.

5. Total product line of TSKgel PW Type

Table 9 lists the total product line of TSKgel PW Type including new series and old series for both analytical and preparative purposes together with guard columns.

Table 9 Total Product Line of TSKgel PW Type

	New Series		Old Series					
	7.8mmI. D. × 300mm		7.5mmI. D. × 300mm		7.5mmI. D. × 600mm		21.5mmI. D. × 600mm	
	Particle size	TP/column	Particle size	TP/column	Particle size	TP/column	Particle size	TP/column
TSKgel G2500PW	6	14,000	10	5,000	10	10,000	17	10,000
TSKgel G3000PW	6	14,000	10	5,000	10	10,000	17	10,000
TSKgel G4000PW	10	10,000	17	3,000	17	6,000	22	6,000
TSKgel G5000PW	10	10,000	17	3,000	17	6,000	22	6,000
TSKgel G6000PW	13	7,000	17	3,000	17	6,000	25	6,000
TSKgel GMPW	13	7,000	17	3,000	17	6,000	—	—
TSKgel G-Oligo-PW	6	14,000	—	—	—	—	—	—
TSKgel G-DNA-PW	10	10,000	—	—	—	—	—	—

	Column systym	Column size
TSKguard Column PW_{XL}	TSKgel $G2500PW_{XL}$ ~ $GMPW_{XL}$	6.0mmI. D. × 90mm
TSKguard Column G-Oligo-PW	TSKgel G-Oligo-PW	6.0mmI. D. × 40mm
TSKguard Column PW	TSKgel G250OPW ~ GMPW	7.5mmI. D. × 75mm
TSKguard Column PW	TSKgel G2500PW ~ G6000PW	21.5mmI. D. × 75mm

Table 10 Range of Elution Conditions for New PW 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	80	10-60	80
TSKgel $G3000PW_{XL}$	0.5-0.8	1.0	80	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	30	10-60	80
TSKgel G-Oligo-PW	0.5-0.8	1.0	60	10-60	80
TSKgel G-DNA-PW	0.2-0.5	0.6	20	10-40	50

It should be noted that there are several modifications in the old series as follows :

(1) Introduction of G250OPW

(2) Introduction of GMPW

(3) Deletion of Gl000PW

6. Some advices for use of PW columns

Detailed description on column maintenance is given in instruction manual for TSKgel PW Columns. Description on the column maintenance for TSKgel SW columns by Watanabe et al[46] is also fundamentally applicable for PW columns.

Here summarized are several points as follows.

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 PW columns.

6-2 Prevention of column deterioration

(1) Use of well filtered solvent and sample solution without fine particles is very important to avoid pressure rise and decrease of performance due to the clogging of the inlet filter and the top of the gel bed.

(2) Protection of the total system from corrosion is also very important to avoid the clogging due to the rust.

(3) A guard column should be used and replaced immediately after any abnormal phenomenon such as pressure rising and decrease of performance is observed.

(4) Keeping flow rate at suitable range instead of maximum serves to avoid bed compression (top-off), resulting in long column life.

(5) Slow and gradual operation during solvent exchange is essential to protect the bed from compression.

7. Matching of column with system

The new PW series should be used with an up-to-date system of satisfactorily small dead volume.

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