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

Hiroo SASAKI
Takao MATSUDA
Osamu ISHIKAWA
Tohru TAKAMATSU
Katsuyuki TANAKA
Yoshio KATO
Tsutomu HASHIMOTO

The outline of the new series of TSK-GEL PW Type is described. Characteristics such as the numbers of theoretical plates, separation ranges and calibration curves are described. Several important basic properties are also described including effect of flow rate, nonexclusion effects, temperature stability, solvent compativility, etc. Applications of the conventional PW columns are reviewed for the best column selection.

The new series consisting of eight grades can cover a wide range of water-soluble substances from small to very large and natural to synthetic. Their performance is greatly improved compared with the conventional PW columns.

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 (60 cm), 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. 4000Å) and small particle size (10 μm) can separate large DNA fragments almost 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 TSKgel PW columns, (2) separation of water-soluble polymers on new TSKgel PW columns, (3) separation of large DNA fragments on TSKgel G-DNA-PW column.

2. Characteristics of new PW 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 (polyethylene 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.

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

Column	Particle Size μm	Theoretical Plate Number Guaranteed (TP/Column)	Exclusion Limit*2			Column Dimmension mmI. D. ×cm
			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	($>4\times10^6$)	
TSKgel G5000PW$_{XL}$	10	10,000	1×10^6	($>2.5\times10^6$)	($>1\times10^7$)	
TSKgel G6000PW$_{XL}$	13	7,000	(2×10^7)	($>5\times10^7$)	($>2\times10^8$)	
TSKgel GMPW$_{XL}$	13	7,000	(2×10^7)	($>5\times10^7$)	($>2\times10^8$)	
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.
Eluent: Distilled water
Flow rate: 1.0 mℓ/min
Sample: Ethylene glycol 1%×20μℓ

*2 The values in parentheses are presumed.

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 TSK-GEL PW columns as shown in **Table 3**.

Figures 1 ~ 3 show the calibration curves for TSKgel PW$_{XL}$ columns measured with the above-mentioned standards, respectively.

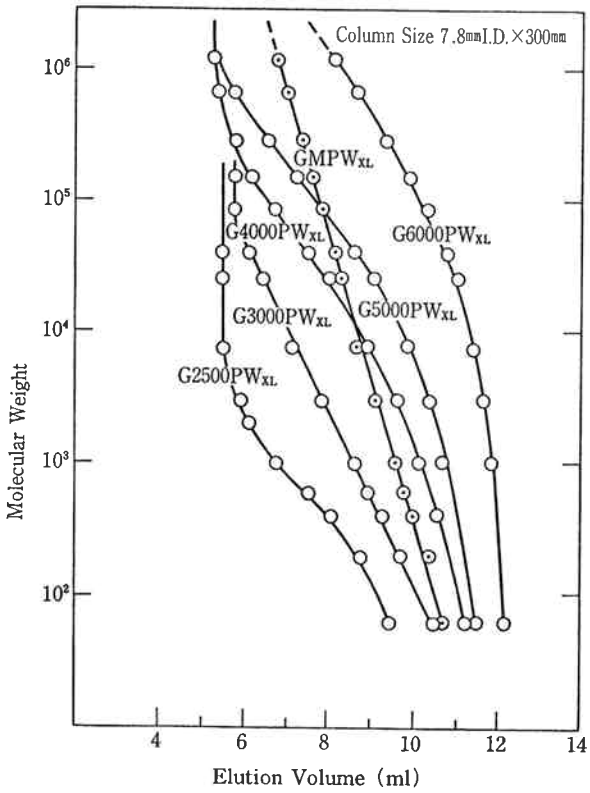
Figure 4 shows the calibration curve for a TSKgel G-Oligo-PW column (solid line) together with the one for TSKgel G2500 column (dotted line) measured with polyethylene glycol standards. The calibration curve of TSKgel G-DNA-PW for double-stranded DNA fragments will be presented elsewhere[42].

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

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

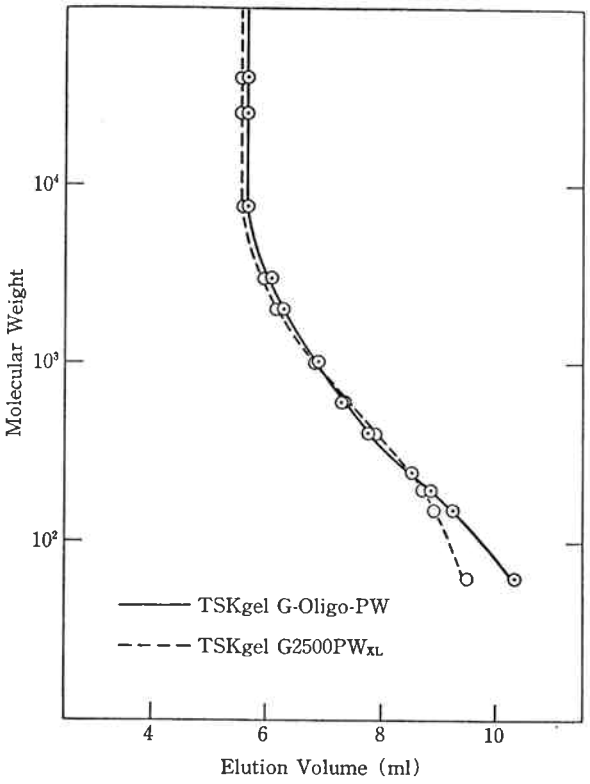
Table 3 Comparison of Theoretical Plate Number Guaranteed between New and Old PW Series

Grade	Old Series		New Series
	7.5mmI. D. ×600 mm	7.5mmI. D. ×300mm	7.8mmI. D. ×300 mm
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



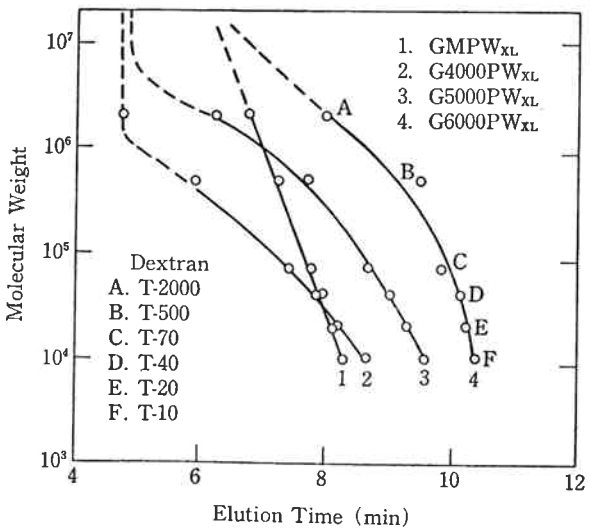
Sample : PEG and PEO Standards
Mobile phase : Distilled water
Flow rate : 1.0 mℓ/min.

Fig. 1 Calibration Curves of TSKgel PW$_{XL}$ Columns for PEG and PEO Standards



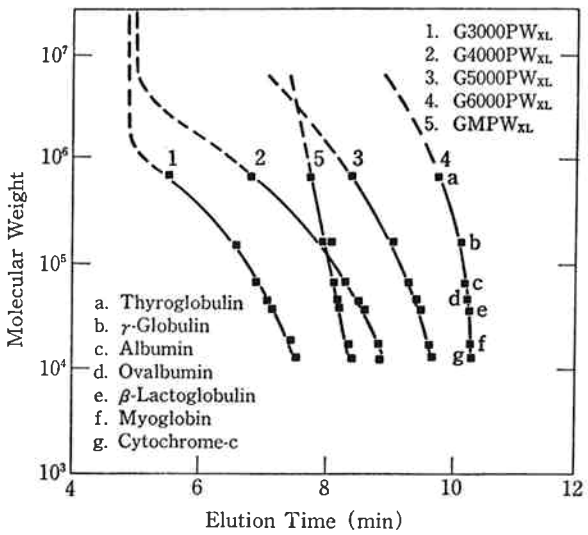
Column size : 7.8 mmI. D. × 30 cm
Sample : PEG and PEO Standards
Mobile phase : Distilled water
Flow rate : 1.0 mℓ/min.

Fig. 4 Calibration Curves of TSKgel G-Oligo-PW and TSKgel G2500PW$_{XL}$



Mobile phase : 0.2 M P.B. (pH 6.8)
Flow rate : 1.0 mℓ/min.

Fig. 2 Calibration Curves of TSKgel PW$_{XL}$ Columns for Dextran Standards



Mobile phase : 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

3. Basic properties of new PW columns

[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 6 μm 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 13 μm 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.

[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 0.1 μ.

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.

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.

[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.

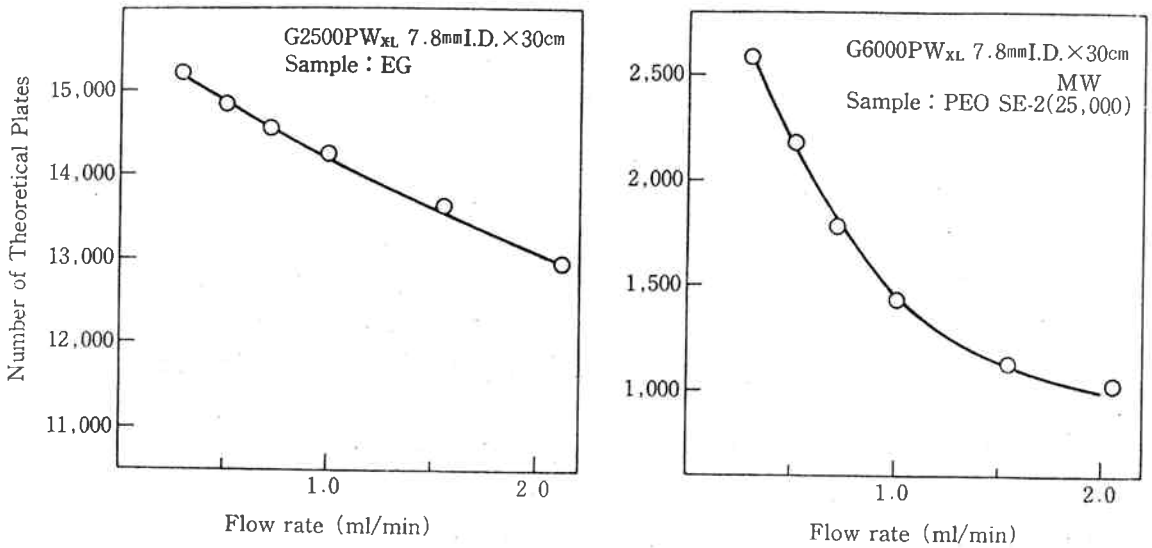


Fig. 5 Flow Rate Dependence of the Number of Theoretical Plates on TSKgel $G2500PW_{XL}$ and $G6000PW_{XL}$

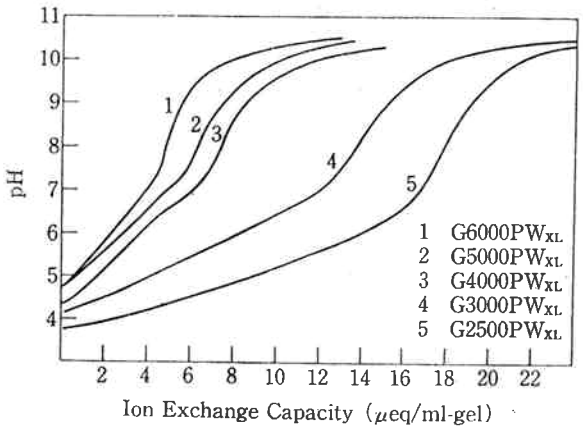


Fig. 6 Titration Curves of PW_{XL} Gels

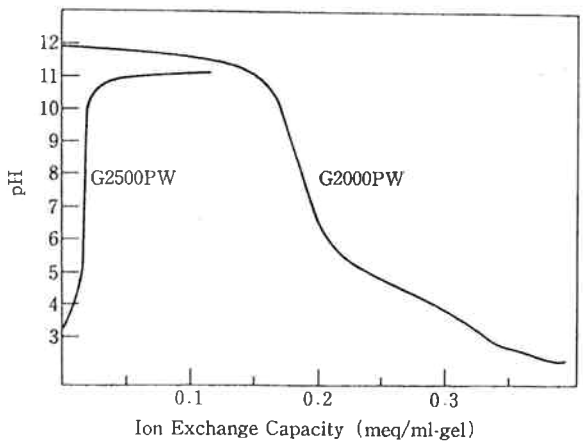
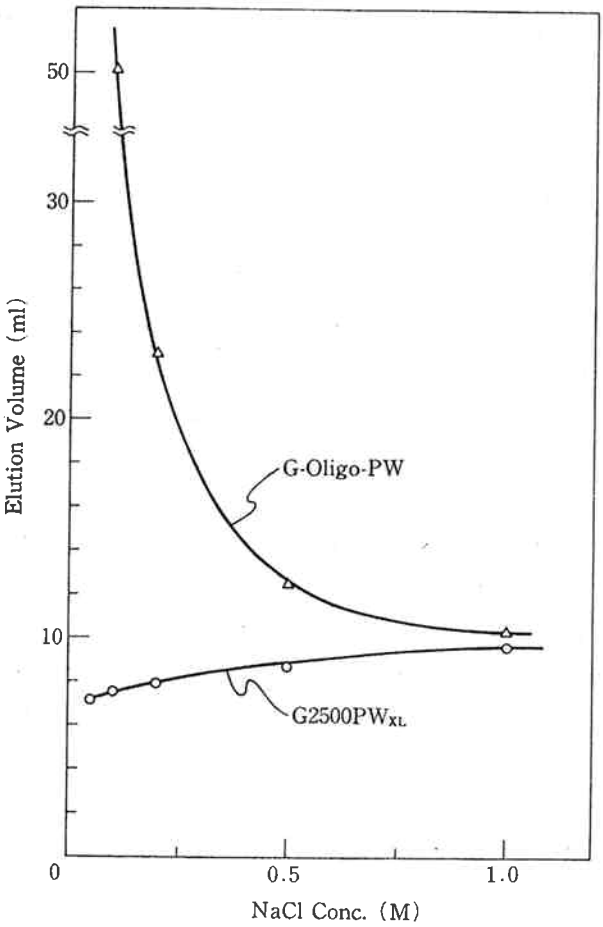


Fig. 7 Comparison of Titration Curves between G2500PW and G2000PW Gels

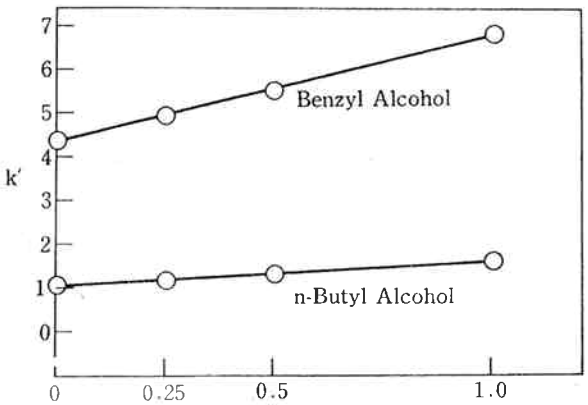


Column size : 7.8 mmI.D. ×30 cm
Sample : Adenosine monophosphate
Mobile phase : 0.02 M P.B. (pH 6.8) + 0.05 M —1.0 M NaCl

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

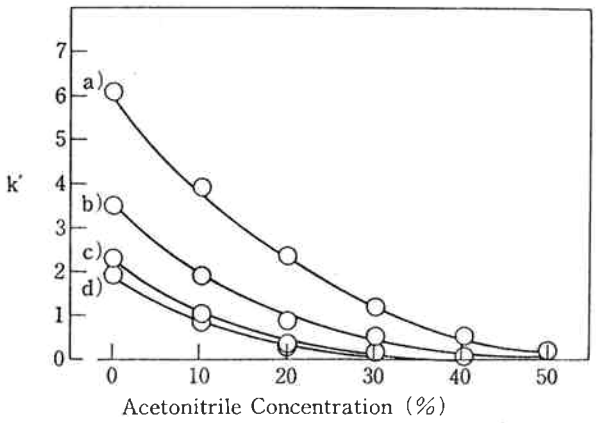
Table 4 Capacity Factors of Aliphatic Alcohols

Column	Ethyl Alcohol	iso-Propyl Alcohol	n-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



Column : TSKgel G2500PW$_{XL}$
Column size : 7.8 mmI. D. × 30 cm
Sample : (1) Benzyl Alcohol
(2) n-Butyl Alcohol
Flow rate : 1.0 mℓ/min.

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 mmI. D. × 30 cm
Sample : (a) β-Phenethyl alcohol
: (b) Adenine
: (c) Adenosine
: (d) Tryptophan
Flow rate : 1.0 mℓ/min.

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

[4] Temperature stability

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

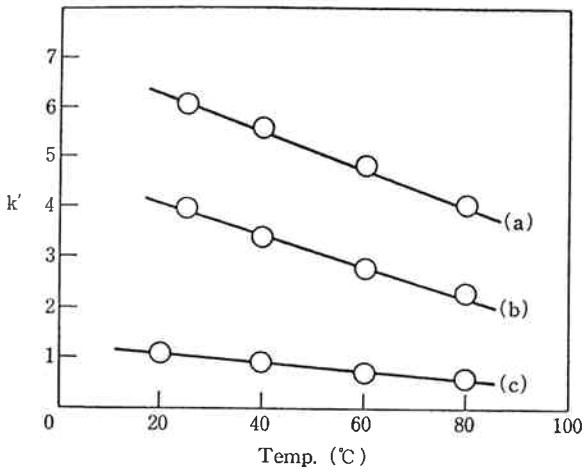
Figure 12 shows an example of a running life test of the columns of TSKgel GMPW$_{XL}$, TSKgel G2500PW$_{XL}$ and TSKgel G-Oligo-PW at 60°C. During the continuous testing of three months, the numbers of theoretical plates and the pressure drops were kept almost constant.

[5] Solvent compatibility

(1) Organic solvent

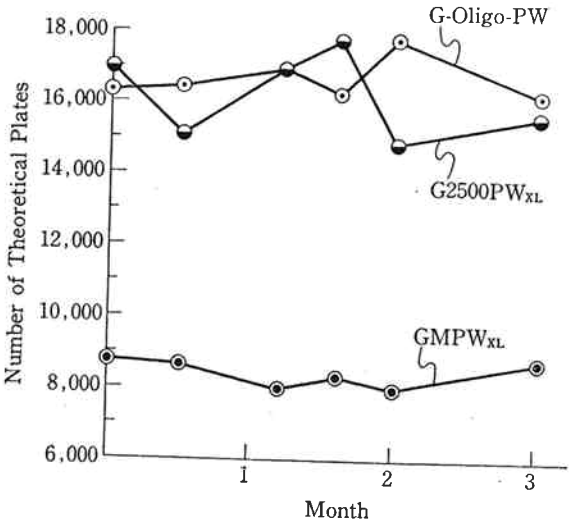
Water-soluble organic solvents are frequently used as a modifier in order to suppress hydrophobic interaction between PW columns and samples. Typical examples are listed in **Table 5**.

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,



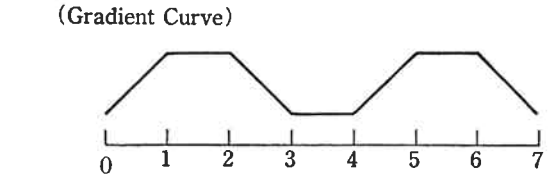
Column : TSKgel G2500PW$_{XL}$
Column size : 7.8 mmI. D. ×30 cm
Sample : β-Phenethyl alcohol
Mobile phase : (a) Water
(b) Acetonitrile 10% Soln.
(c) Acetonitrile 30% Soln.
Flow rate : 1.0 mℓ/min.

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



Column size : 7.8 mmI. D. ×30 cm
Sample : Ethylene glycol
(Condition)
Running Condition;
Flow rate : 1.2 mℓ/min.
Temp. : 60°C
Measuring Condition;
Flow rate : 1.0 mℓ/min.
Temp. : 25°C

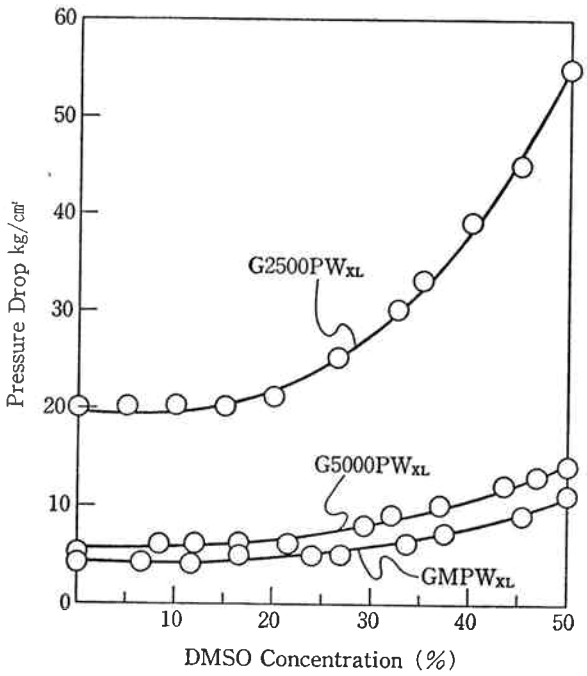
Fig. 12 An Example of Column Life Test at 60°C



H_2O	100	50	50	100	100	50	50	100(%)
Solvent	0	50	50	0	0	50	50	0 (%)

Condition : Organic Solvents from Water, 60 min. Linear gradient
Flow rate : 0.5 mℓ/min.

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



Column : (1) TSKgel G2500PW$_{XL}$
(2) TSKgel G5000PW$_{XL}$
(3) TSKgel GMPW$_{XL}$
Column size : 7.8 mmI. D. ×30 cm
Flow rate : 0.5 mℓ/min.

Fig. 14 Relation between Eluent Composition and Pressure Drop

acetone etc.

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 0.5 mℓ/min) with linear gradient according to the procedure in **Figure 13**. Typical examples of the change of the

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	0.1 M Sodium Acetate Containing 20% CH_3CN	14
3	Poly (styrene sulfonate)	G6000PW + G3000PW	0.2 M Phosphate Buffer Containing 10% CH_3CN	14
4	Poly (dimethyl aminoethyl methacrylate)	G6000PW + G3000PW	0.5 M Sodium Acetate Containing 0.5 M Acetic Acid	14
5	Poly (ethyleneimine)	G6000PW + G3000PW	0.5 M Sodium Acetate Containing 0.5 M Acetic Acid	14
6	Chitosan	G6000PW + G3000PW	0.5 M Sodium Acetate Containing 0.5 M Acetic Acid	14
7	Glycol chitosan	G5000PW + G3000PW	0.3 M Sodium Sulfate Containing 0.5 M Acetic Acid	14
8	Poly (4-vinyl benzyl trimethyl ammonium chloride)	G5000PW + G3000PW	0.1 M 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 2) 15,100 3) 14,800	1) 14,700 2) 15,200 3) 15,000	1) 15,600 2) 15,300 3) 14,200	1) 16,600 2) 18,000 3) 17,400
$G3000PW_{XL}$	1) 17,200 2) 16,500 3) 16,200	1) 16,000 2) 16,500 3) 15,700	1) 18,300 2) 19,100 3) 18,600	1) 18,000 2) 18,500 3) 18,700
$G4000PW_{XL}$	1) 13,100 2) 13,700 3) 13,300	1) 12,900 2) 12,700 3) 13,000	1) 12,600 2) 12,800 3) 12,500	1) 13,000 2) 12,700 3) 13,200
$G5000PW_{XL}$	1) 12,400 2) 11,000 3) 11,800	1) 13,000 2) 12,500 3) 12,300	1) 12,400 2) 12,000 3) 11,800	1) 13,700 2) 13,700 3) 13,900
$G6000PW_{XL}$	1) 7,800 2) 7,300 3) 8,200	1) 8,800 2) 8,100 3) 8,400	1) 8,000 2) 7,800 3) 7,800	1) 8,800 2) 8,800 3) 8,200
$GMPW_{XL}$	1) 7,600 2) 6,900 3) 7,500	1) 7,700 2) 7,400 3) 7,800	1) 7,200 2) 8,100 3) 7,300	1) 7,400 2) 6,600 3) 7,600
G-Oligo-PW	1) 16,200 2) 17,100 3) 16,900	1) 17,200 2) 17,400 3) 16,900	1) 16,400 2) 16,000 3) 16,100	1) 14,800 2) 15,200 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.

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 pH[12] and low pH[2] at room temperature.

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

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 TSK-GEL SW Type, seven of the conventional TSK-GEL PW Type and eight of the new series of TSK-GEL 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.

[1] Which is better PW or 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 columns 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 substances 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 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

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$_{XL}$		