TOSOH

SEPARATION REPORT NO. 38

GFC SEPARATION OF WATER-SOLUBLE POLYMERS USING TSKgel PWXL SERIES COLUMNS

Table of Contents

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.

Contact us today for more information

EMEA +49 6155 704 37 12
techsupport.tbg@tosoh.com
USA +1-800-366-4875 option #3
techservice.tbl@tosoh.com
www.tosohbioscience.com

1. Introduction

High performance gel permeation (GPC) and gel filtration chromatography (GFC) are widely used separation modes due to their separation power, good reproducibility, and short analysis time.

As shown in Table 1, which indicates the decades during which several high performance packing materials for GPC and GFC were developed, Tosoh has been in the forefront of developing high performance columns for both organic (GPC) and aqueous (GFC) solvent systems. To satisfy the demand for faster, more sensitive and labor-saving analytical equipment, Tosoh scientists and engineers continue to develop smaller particle size, higher performance, and faster columns for both organic and aqueous solvent systems.

This report discusses the separation performance of the high performance TSKgel PW_{XL} series of GFC columns for use in aqueous systems and presents applications for using the TSKgel PW_{XL} columns.

Table 1 History of development of GPC and GFC packing materials

		Historical background
1970	Development of TSKgel S- and H-type GPC packing materials for organic solvent systems	Petrochemical industry is flourishing
1975	Development of TSKgel SW- and PW-type packing materials for aqueous solvent systems (GFC)	Oil shock Growth of high value-added industries
1980	Development of Toyopearl® packing materials for preparative GFC and other modes of chromatography	Start of the biotech revolution
1987	Development of packing materials for GPC and GFC with high theoretical plate numbers	Analytical equipment becoming faster, more sensitive, and more labor saving

2. Calibration curves, theoretical plate numbers, and separation range for each grade in the TSKgel PW_{XL} series columns

Figure 1 shows calibration curves for each column in the TSKgel PW_{XL} series using polyethylene oxide (PEO) SE-series standards produced by Tosoh Corporation. A TSKgel $GMPW_{XL}$ column is a mixed bed column that is prepared by mixing batches of particles each containing a narrow but different pore size distribution, making this column suitable for analyzing molar mass and molar mass distributions. A TSKgel $G2500PW_{XL}$ column has also been added to the TSKgel PW_{XL} series columns. In this column, residual negative charges of the conventional TSKgel G2000PW column have been reduced while the resolution maintained (for details see Separation Report 037).

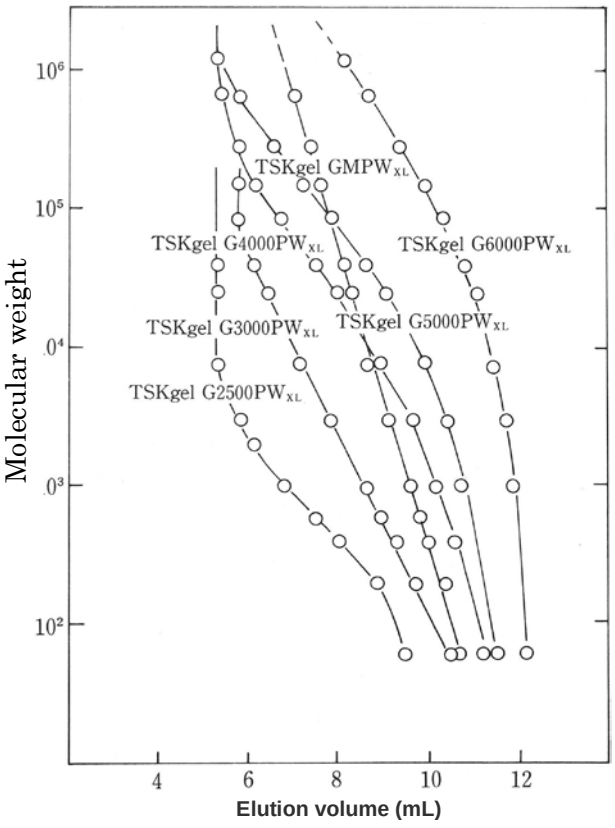
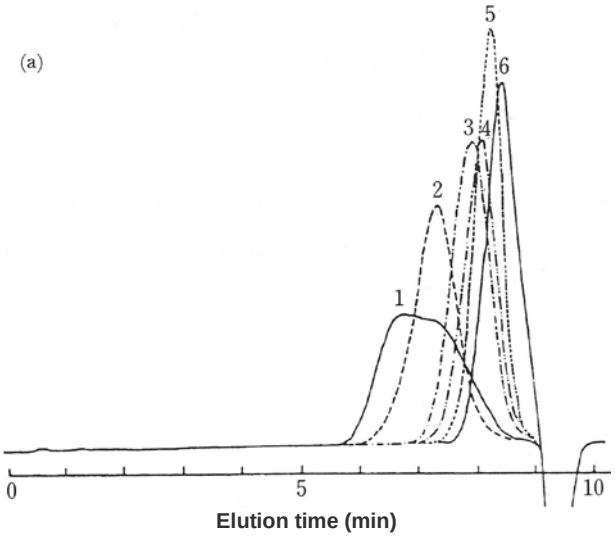


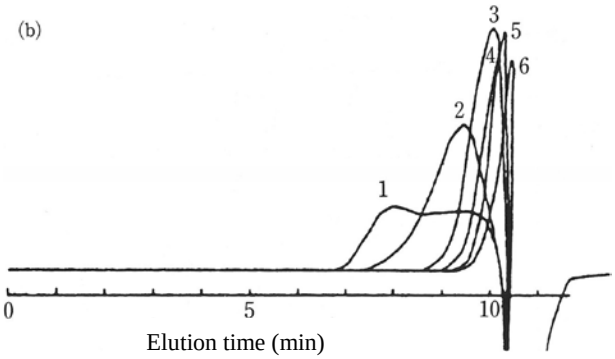
Figure 1 Calibration curves for the PW_{XL} columns

Columns: 7.8mm ID x 30cm
Sample: polyethylene oxide standards

Table 2 lists names, particle size, theoretical plate number, and molar mass exclusion limit of each column within the TSKgel PW_{XL} series. Figures 2a-2d show chromatograms produced by separating dextran samples using the TSKgel $GMPW_{XL}$, TSKgel $G6000PW_{XL}$, TSKgel $G5000PW_{XL}$, and TSKgel $G4000PW_{XL}$ columns. Consult Figures 1 and 2 and Table 2 to choose the optimum column system for the sample to be analyzed.



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



Column: TSKgel $G6000PW_{XL}$, 7.8mm ID × 30cm

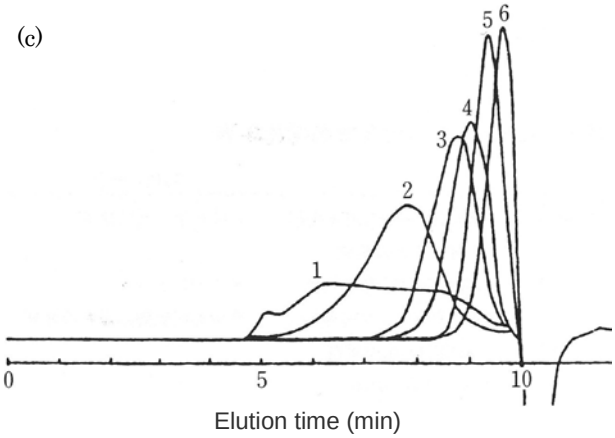
Figure 2 Separation of dextran

Eluent: 0.2mol/L phosphate buffer, pH 6.9
Flow rate: 1.0mL/min
Detection: RI
Temperature: 40°C
Injection vol.: 100µL (1.0g/L)
Samples: 1 Dextran T-2000
2 Dextran T-500
3 Dextran T-70
4 Dextran T-40
5 Dextran T-20
6 Dextran T-10

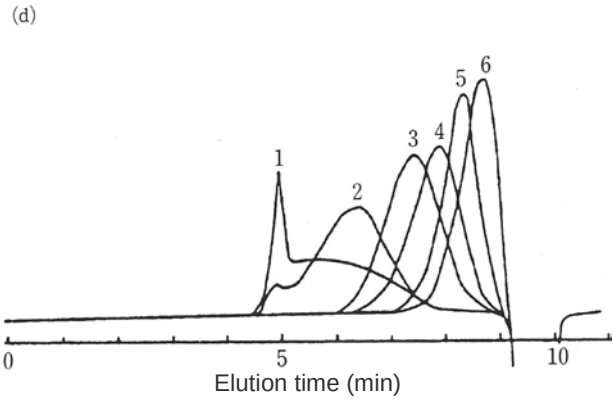
Table 2 TSKgel PW_{XL} Columns

Name	Particle size (µm)	Theoretical plate number	Exclusion limit (Da)
$G2500PW_{XL}$	7	14,000	10,000
$G3000PW_{XL}$	7	14,000	100,000
$G4000PW_{XL}$	10	10,000	1,000,000
$G5000PW_{XL}$	10	10,000	4,000,000
$G6000PW_{XL}$	13	7,000	—
$GMPW_{XL}$	13	7,000	—
G-Oligo-PW	7	14,000	10,000
G-DNA-PW	10	—	—

(Each column: 7.8mm ID × 30cm)



Column: TSKgel $G5000PW_{XL}$, 7.8mm ID × 30cm

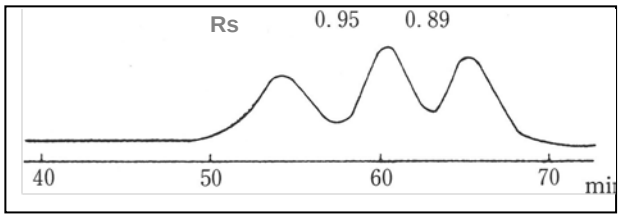


Column: TSKgel $G4000PW_{XL}$, 7.8mm ID × 30cm

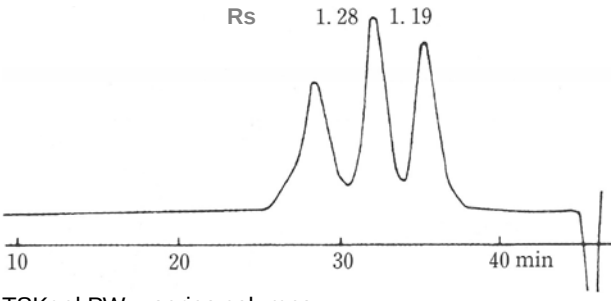
3. Comparison of performance of TSKgel PW_{XL} and PW columns

Figures 3 and 4 are chromatograms comparing the performance of a TSKgel G6000PW + TSKgel G3000PW series of columns and a TSKgel G6000PW_{XL} + TSKgel G3000PW_{XL} series of columns.

Figure 3 compares elution profiles and resolution (Rs) obtained using a sample consisting of polyethylene oxide standards (SE-150: 1.20 $\times 10^6$ Da; SE-30: 2.8 $\times 10^6$ Da; SE-8: 7.3 $\times 10^4$ Da).



TSKgel PW series columns
Column size: 7.5mm ID × 60cm × 2



TSKgel PW_{XL} series columns
Column size: 7.8mm ID × 30cm × 2

Figure 3 Comparison of TSKgel PW and PW_{XL} Columns (1)

Column: (Top) TSKgel G6000PW + TSKgel G3000PW
(Bottom) TSKgel G6000PW_{XL} + TSKgel G3000PW_{XL}

Eluent: 0.1mol/L NaCl
Flow rate: 0.5mL/min
Temperature: 50°C
Injection vol.: 100µL (0.4g/L)
Sample: polyethylene oxide, SE-150, SE-30, SE-8

Figure 4 compares elution profiles obtained using a mixture of pullulan standards (P-400: 3.38 $\times 10^5$ Da; P-50: 4.67 $\times 10^4$ Da) as the sample. Columns in the TSKgel PW_{XL} series are half as long as the 60cm columns in the TSKgel PW series columns, thus separation is completed in half the time, while resolution is equivalent to or higher than results produced with the TSKgel PW-type columns.

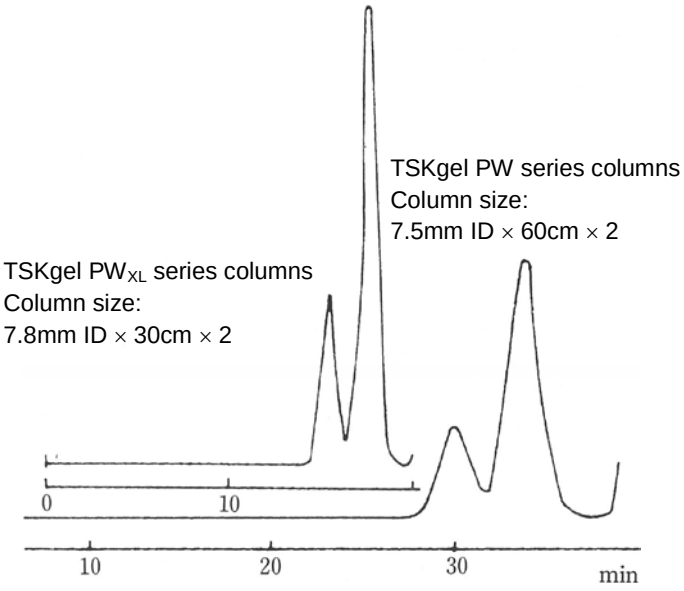


Figure 4 Comparison of TSKgel PW and PW_{XL} Columns (2)

Column: (A) TSKgel G6000PW_{XL} + TSKgel G3000PW_{XL}
(B) TSKgel G6000PW + TSKgel G3000PW

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 50°C
Injection vol.: 100µL (0.4, 1.2g/L)
Sample: pullulan, P-400-P-50

Figure 5 shows the results of a comparison of the performance of two TSKgel PW_{XL} columns with that of two TSKgel PW columns. A mixture of polyethylene oxide standards (SE-150: 1.20×10^6 Da; SE-15: 15×10^4 Da; SE-2: 2.5×10^4 Da) was used as the sample. As in Figures 3 and 4, performance of the TSKgel PW_{XL} columns is the same or better at half the analysis time.

4. Effect of elution conditions on resolution of the TSKgel PW_{XL} series

1) Flow rate

Figure 6 compares elution profiles at flow rates of 1.0mL/min and 0.5mL/min. Resolution (Rs) between the peaks are noted on the chromatograms. As expected according to theory, resolution improves as the flow rate decreases.

2) Temperature

Figure 7 compares the elution profiles at 25°C and 50°C using a constant flow rate of 0.5mL/min. Increasing temperature causes a significant improvement in the resolution between the peaks. Note that the retention times do not change in size exclusion chromatography as it is an entropy-driven process.

3) Sample concentration

Figure 8 compares elution profiles produced when the sample concentration of each component was 0.4g/L versus 1.6g/L. Elution profiles are sensitive to sample volume used and total sample mass injected. Above a certain injection volume and sample mass, efficiency decreases due to extra-column band broadening due to mass overload. For example, in Figure 8, the sample mass injected (at 1.6g/L for each component) causes band broadening that was not visible at lower mass load.

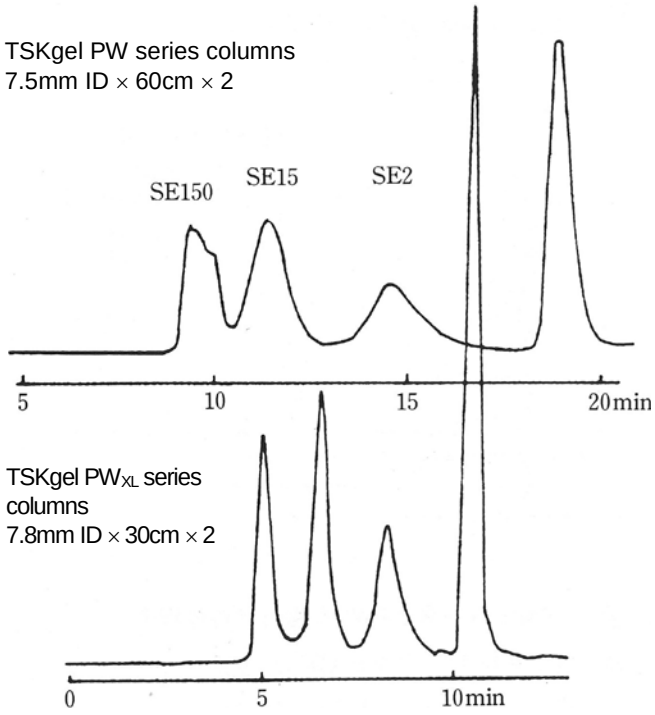


Figure 5 Comparison of TSKgel PW and PW_{XL} Columns (3)

Column:	(A) TSKgel G4000PW (B) TSKgel G4000PW_{XL}
Eluent:	0.1mol/L NaCl
Flow rate:	1.0mL/min
Temperature:	50°C
Injection vol.:	100µL
Sample:	polyethylene oxide standards

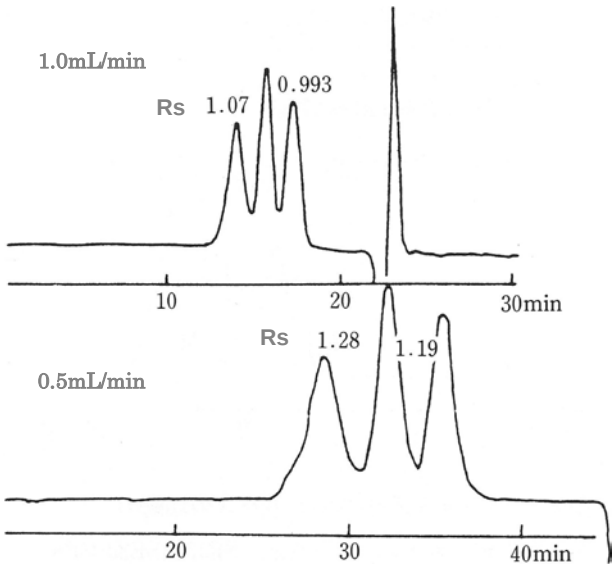


Figure 6 Effect of flow rate

Columns:	TSKgel G6000PW_{XL} + TSKgel 3000PW_{XL}, 7.8mm ID × 30cm × 2
Eluent:	0.1mol/L NaCl
Temperature:	50°C
Injection vol.:	100µL (0.4g/L)
Sample:	polyethylene oxide standards, SE-150, SE-30, SE-8

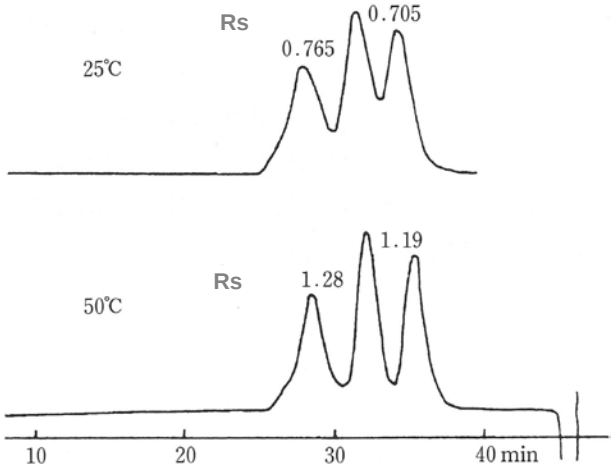


Figure 7 Effect of temperature

Columns: TSKgel G6000PW$_{XL}$ + TSKgel G3000PW$_{XL}$, 7.8mm ID × 30cm × 2

Eluent: 0.1mol/L NaCl
Flow rate: 0.5mL/min
Injection vol.: 100µL (0.4g/L)
Sample: standard polyethylene oxide, SE-150, SE-30, SE-8

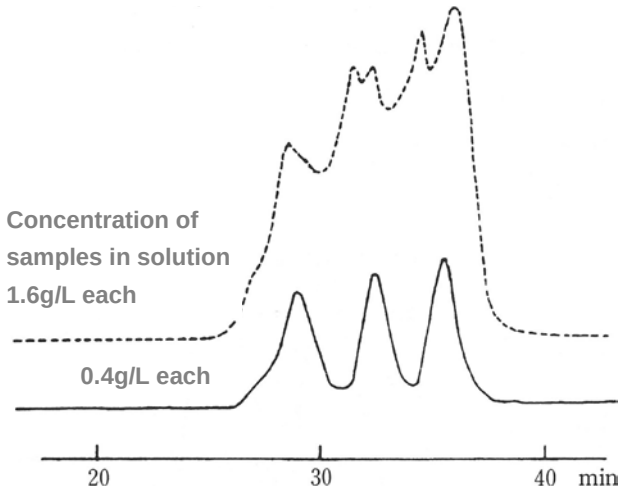


Figure 8 Effect of sample concentration in solution

Columns: TSKgel G6000PW$_{XL}$ + TSKgel G3000PW$_{XL}$, 7.8mm ID × 30cm × 2

Eluent: 0.1mol/L NaCl
Flow rate: 0.5mL/min
Temperature: 50°C
Injection vol.: 100µL (0.4g/L each and 1.6g/L each)
Sample: standard polyethylene oxide, SE-150, SE-30, SE-8

4) Sample injection volume

Figure 9 compares the elution profiles produced with sample injection volumes of 100µL and 500µL. At the same sample load, the degree of change in Rs is less when the injection volume is increased without changing the concentration of the sample in the solution, compared to when the concentration of the sample in the solution is increased without changing the injection volume (see Figures 8 and 9). When analysis sensitivity is low, increasing the injection volume improves precision. For reference, Figure 10 illustrates the effect of injection volume on elution profiles with the TSKgel PW columns. The TSKgel PW$_{XL}$ columns are more sensitive to injection volume, as well as the total mass injected, than the TSKgel PW columns because the column volume of the 30cm columns is half of that of the 60cm columns and the efficiency of TSKgel PW$_{XL}$ columns is higher, i.e. peak volumes are smaller.

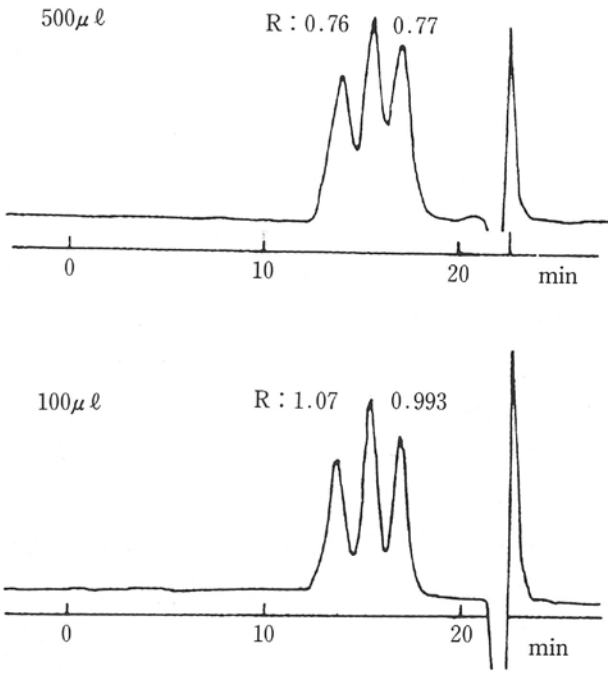


Figure 9 Effect of sample injection volume (1)

Columns: TSKgel G6000PW$_{XL}$ + TSKgel G3000PW$_{XL}$, 7.8mm ID × 30cm × 2

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 50°C
Sample: standard polyethylene oxide, SE-150, SE-30, SE-8
Sample concentration: 0.4g/L each

5. Relationship between theoretical plate number and resolution (Rs)

In high performance liquid chromatography, the quality of the separation between components is expressed by Equation (1).

$$Rs = \frac{1}{4}\sqrt{N}\left(\frac{\alpha-1}{\alpha}\right)\cdot\left(\frac{K'}{K'+1}\right) \quad \cdots\cdots (1)$$

Here Rs represents the resolution of two peaks, with better separation indicated by larger Rs values. N expresses the theoretical plate number; α, the selectivity factor, and K´, the retention factor, which are calculated using Equations (2) and (3), respectively.

$$\alpha = \frac{V_{R2}-V_O}{V_{R1}-V_O} \quad \cdots\cdots (2)$$

$$K' = \frac{V_R-V_O}{V_O} \quad \cdots\cdots (3)$$

V_R and V_O are the retention volume of the analyte and the void volume, respectively. The method for calculating the resolution Rs is shown in Figure 11.

Due to (ideally) the absence of interaction between the sample and the packing material in GFC and GPC, α and K´ are constants that are unrelated to the length of the column.

Thus Equation (1) becomes:

$$Rs = K\sqrt{N} \quad \cdots\cdots (1') \quad K: \text{constant value}$$

A simple way to increase resolution is to increase the number of theoretical plates by lengthening the column.

Figure 12 shows changes in the elution profiles for a mixture of polyethylene oxide standards when changing the length of the column. As expected from equation 1, resolution improves as the length of the column increases. Table 3 shows theoretical plate number N for SE-15 at various column lengths as well as calculations of Rs between peaks SE-15 and SE-2. Rs values are plotted as a function of $\sqrt{N}$ in Figure 13. A very good linear relationship is achieved, satisfying the relationship expressed by equation (1').

6. Dependence of M_w/M_n and M_z/M_w on theoretical plate number and sample concentration in GFC

M_w/M_n and M_z/M_w values for polyethylene oxide SE-15, pullulan P-50 and P-400, and dextran T-70 standards analyzed on TSKgel G4000PW$_{XL}$ columns with varying column lengths are shown in Figure 14. M_w/M_n and M_z/M_w values for the dextran, pullulan, and polyethylene oxide standards reached a constant value for a column length of ≥120cm.

Figure 15 shows changes in M_w/M_n and M_z/M_w values produced when the concentration of the sample was varied using a 30cm column. M_w/M_n and M_z/M_w values did not change over the concentration range of 0.05-0.2% (injection volume: 100µL).

For each of these samples, M_w/M_n and M_z/M_w values approach unity as the column length, thus theoretical plate numbers, increase. However, to obtain accurate M_w/M_n and M_z/M_w values, a column length of 120cm is necessary when using a TSKgel G4000PW$_{XL}$ column.

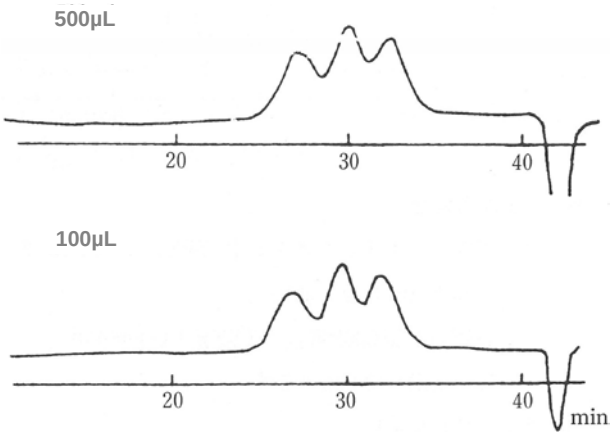


Figure 10 Effect of sample injection volume (2)

Columns: TSKgel G6000PW + TSKgel G3000PW, 7.5mm ID × 60cm × 2

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 50°C
Sample: standard polyethylene oxide, SE-150, SE-30, SE-8
Sample solution concentration: 0.4g/L each

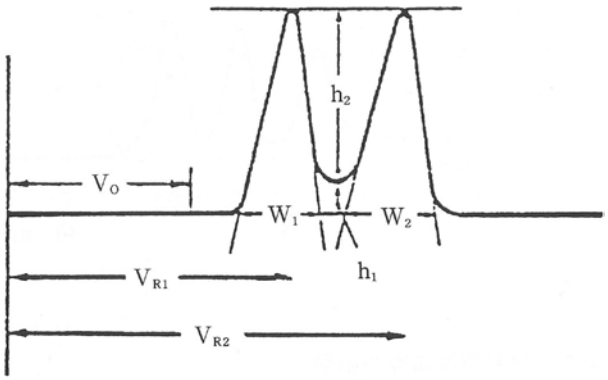


Figure 11 Method for calculating resolution factor

$$Rs = \frac{2\,(V_{R_2} - V_{R_1})}{W_1 + W_2} \quad \cdots\cdots \text{(a) or:}$$

$$Rs = \left[\frac{h_2}{h_1 + h_2}\right] \times 100 \quad \cdots\cdots \text{(b)}$$

Note to Figure 11: The value of Rs in equation (a) increases as separation improves, while in (b) Rs will reach a maximum value of 100% when baseline separation is achieved.

However, currently the most problematic area of GFC with aqueous systems is that it is not possible to obtain a series of standard samples with a molar mass of 1 million or higher. This often becomes inconvenient for calculating M_n, M_w and M_z, and development of a series of standard samples with high molar mass is needed for use in aqueous systems.

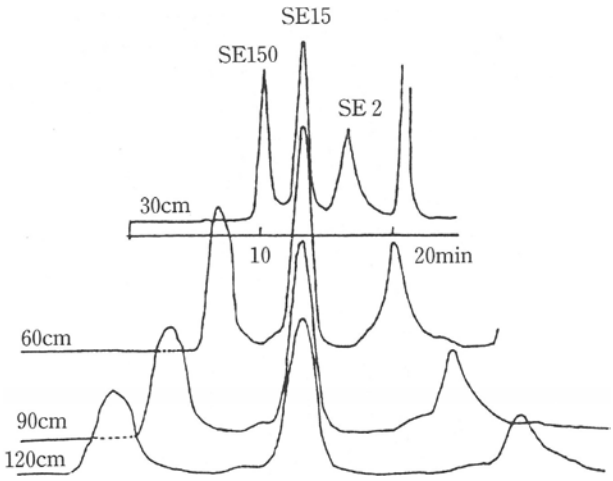


Figure 12 Dependence of changes in separation on column length

Column: TSKgel G4000PW$_{XL}$, 7.8mm ID

Eluent: 0.1mol/L NaCl
Flow rate: 0.5mL/min
Temperature: 50°C
Injection vol.: 100µL (0.4g/L each)
Sample: standard polyethylene oxide, SE-150, SE-15, SE-2

Table 3 Theoretical plate number and resolution factor

Column length (cm)	N*	$\sqrt{N}$	Rs**
30	800	28.3	1.476
60	1900	43.6	2.444
90	2930	54.1	3.04
120	4675	68.4	3.69

*N: Theoretical plate number calculated from peak for SE-15.

**R: Resolution factor between peaks for SE-15 and SE-2.

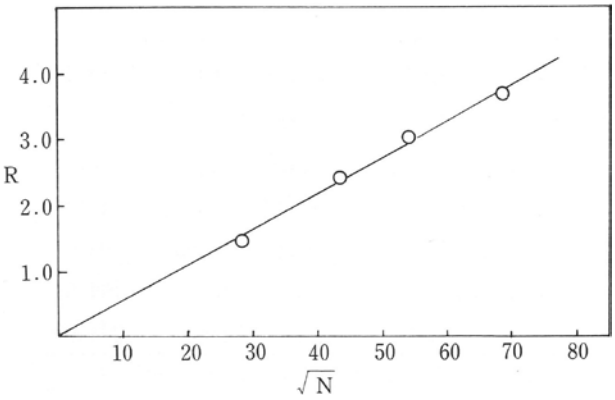


Figure 13 Theoretical plate number and resolution

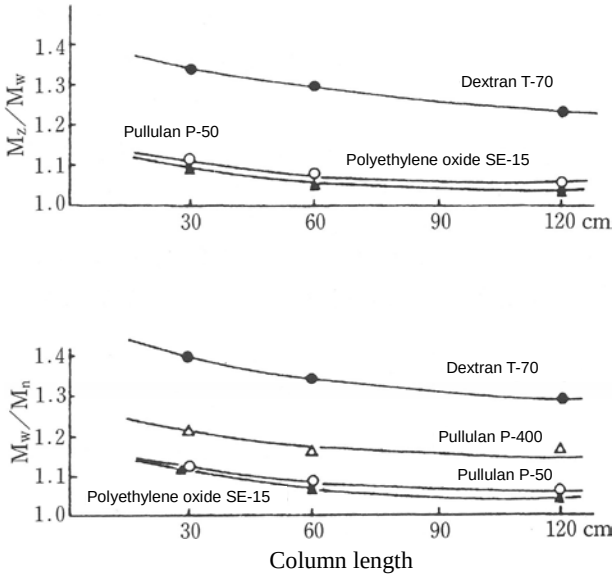


Figure 14 Dependence of M_w/M_n and M_z/M_w values on theoretical plate number

Column: TSKgel G4000PW$_{XL}$, 7.8mm ID

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 50°C
Injection vol.: 100µL (1.0g/L each)

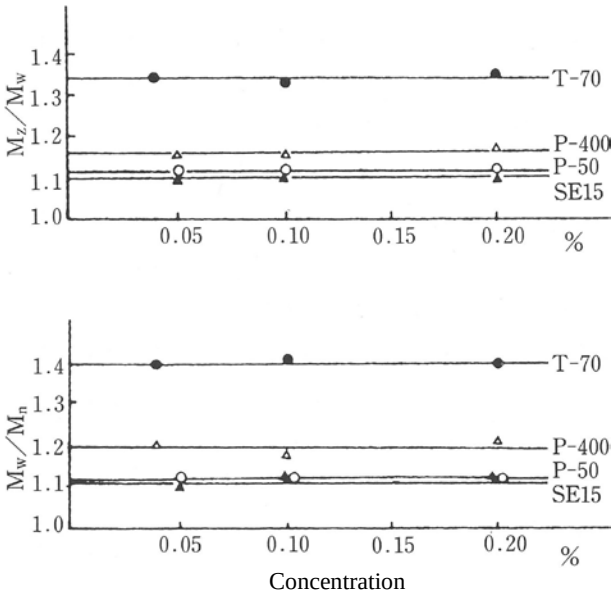


Figure 15 **Dependence of M_w/M_n and M_z/M_w values on concentration of sample solution**

Column: TSKgel G4000PW$_{XL}$, 7.8mm ID × 30cm

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 50°C
Injection vol.: 100µL

7. Analyzing molar mass by GFC/LALLS (low-angle laser light scattering detector) using a TSKgel GMPW$_{XL}$ column

1) Analyzing weight-average molar mass M_w of pullulan standard sample

Pullulan standard samples with a narrow molar mass distribution are commercially available. The molar mass of pullulan was analyzed by GFC/LALLS using a TSKgel GMPW$_{XL}$ column (Figure 16).

Table 4 shows the results of separation for each pullulan standard. Human serum albumin (M=66,000) was used as the primary standard, with pullulan P-100 as the secondary standard. For pullulan, molar masses were determined by Professor Kawahara et al., using sedimentation equilibrium and by Professor Takagi et al., using GFC/LALLS

2) Measuring the weight-average molar mass M_w of standard sodium polystyrene sulfonate (PNaSS)

Sodium polystyrene sulfonate standards are commercially available from Pressure Chemical Company (Pittsburgh, PA), as a standard polymer electrolyte with a narrow molar mass distribution.

Separation of sodium polystyrene sulfonate standards by GFC requires the addition of at least 10% acetonitrile or methanol to a 0.2mol/L Na_2SO_4 mobile phase. Figure 17 shows chromatograms for sodium polystyrene sulfonate standards using a TSKgel GMPW$_{XL}$ column. Peak shapes for sodium polystyrene sulfonate samples obtained by adding 10% acetonitrile to a 0.2mol/L Na_2SO_4 mobile phase remained constant upon addition of more acetonitrile

Table 5 shows the results of a study of the differences in the apparent molar mass M_w of each sample with varying eluent compositions. Small changes in the concentration of inorganic salt or % acetonitrile in the mobile phase caused only minor changes in apparent M_w. However, the values resulting from GFC analyses in each case were slightly higher than the nominal values provided by the manufacturer. Various explanations for this have been considered, including the complexity of the solvent composition, the inability of this method to handle light scattering in a two-component solution, and the hygroscopic properties of the samples. This problem will be a subject of future research in GFC/LALLS.

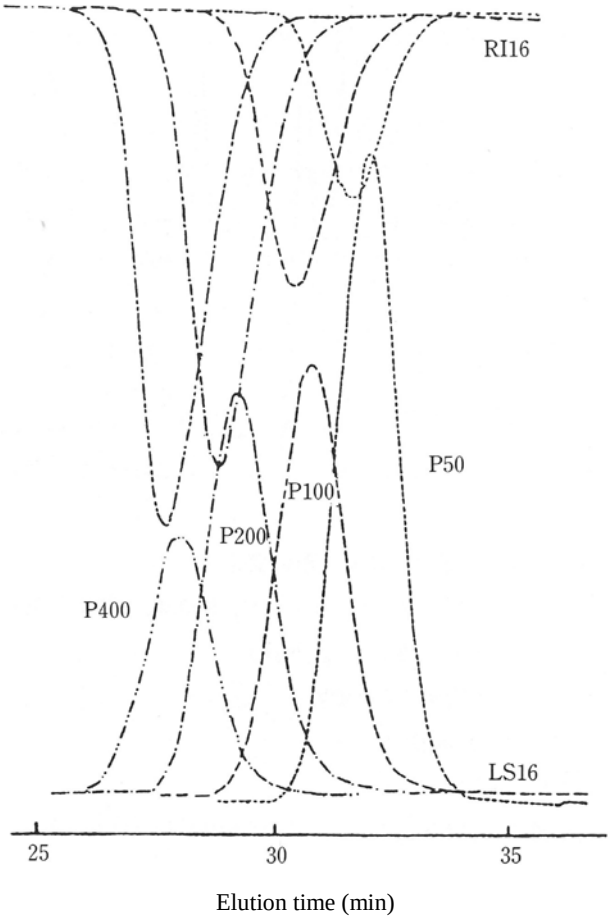


Figure 16 **Chromatogram of pullulan by GFC/LALLS**

Column: TSKgel GMPW$_{XL}$, 7.8mm ID × 120cm

Eluent: 0.1mol/L NaCl
Flow rate: 1.0mL/min
Temperature: 40°C
Injection vol.: 500µL
Sample: pullulan

Table 4 Results of pullulan molar mass analysis

Pullulan Standards	M_w	Ref. 1	Ref. 2
P-10	10,100	10,400	12,000
P-20	18,600	18,200	20,800
P-50	45,700	45,500	46,700
P-100	98,800	100,000	95,400
P-200	177,500	187,000	194,000
P-400	334,000	348,000	338,000

Ref.1 GFC/LALLS data from Professor Takagi et al.
Ref.2 Sedimentation equilibrium data from Professor Kawahara et al.

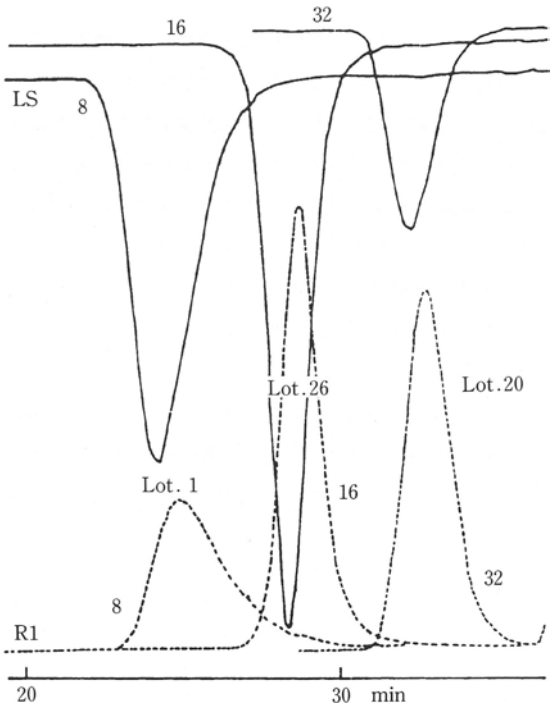


Figure 17 Separation of sodium polystyrene sulfonate standards

Column: TSKgel GMPW$_{XL}$, 7.8mm ID × 30cm × 4

Eluent: 10% acetonitrile/0.2mol/L Na_2SO_4
Flow rate: 1.0mL/min
Temperature: 40°C
Injection vol.: 500µL
Sample: sodium polystyrene sulfonate standard

Table 5 Analysis of weight-average molar mass of standard sodium polystyrene sulfonate by GFC/LALLS

	Apparent M_w			
Lot. No.	Solvent a	b	c	Nominal value
#1	1,210,000	1,210,000	1,290,000	1,060,000
#16	794,000	787,000	821,000	690,000
#26	202,500	202,000	210,000	177,000
#20	19,400	21,000		16,000

Solvents
a: 10% CH_3CN/0.2mol/L Na_2SO_4
b: 20% CH_3CN/0.2mol/L Na_2SO_4
c: 10% CH_3CN/0.4mol/L Na_2SO_4

8. Dependence of M_n, M_w, and M_z on theoretical plate number using GFC/LALLS

Table 6 shows changes in M_n, M_w, and M_z when analysis was performed by GFC/LALLS at varying TSKgel GMPW$_{XL}$ column lengths, using dextran T-500 as the sample. Values in parentheses are without function approximation. (The necessity and validity of functional approximation is published in a separate report.) M_n, M_w, and M_z values do depend slightly on the theoretical plate number of the column or column set. (As in normal GFC, a column with a certain capacity for high resolution separation is necessary to evaluate M_n and M_z.)

Table 6 Analysis of weight-average molar mass by GFC/LALLS

Column length (cm)	$M_n \times 10^4$	$M_w \times 10^4$	$M_z \times 10^4$
60	20.6 (19.7)	43.2 (44.2)	123.4 (139.0)
120	19.5 (20.2)	44.1 (44.1)	139.8 (140.5)
240	19.3 (19.1)	45.4 (45.1)	143.5 (139.0)

Values in parentheses were calculated without functional approximation.

Column: TSKgel GMPW$_{XL}$
Eluent: 0.1mol/L NaCl
Sample: Dextran T-500

9. Selection of solvents for GFC separation of water-soluble polymers

Table 7 lists various types of common water-soluble polymers. Although there are many types of water-soluble polymers, most samples can be analyzed using a limited number of mobile phases as is indicated in Table 8. For example, analyzing viscose is difficult, as this substance can only be dissolved in a highly alkaline aqueous solution. Also, to analyze polymers with aromatic rings in their side chain, such as sodium polystyrene sulfonate, requires the addition of methanol or another organic solvent, to reduce hydrophobic interaction with the particle matrix.

To date, the separation of polycations is challenging due to the presence of trace quantities of carboxyl groups in the particle matrix. However, this problem can be solved by working at an acidic pH, thus inhibiting the dissociation of carboxyl groups and by increasing the ionic strength. It should be noted that when polyanions are analyzed after having performed the separation of a polycation sample on the same column, polyanions, which are usually easy to analyze, tend to show signs of adsorption. Therefor it is recommended to dedicate a column strictly for the separation of polycation samples.

Several GFC applications of water-soluble polymers are shown in Figures 18-23. Figures 18-21 are chromatograms using a TSKgel G6000PW$_{XL}$, TSKgel G5000PW$_{XL}$, and TSKgel G4000PW$_{XL}$ column, respectively. Figure 22 shows the results using the TSKgel GMPW$_{XL}$ column. Figure 23 shows various polycations in an acetate buffer system using the TSKgel GMPW$_{XL}$ column. Each of these chromatograms was produced at a flow rate of 1.0mL/min with RI detection.

Note: Consult the Tosoh Bioscience website for TSKgel PW$_{XL}$-CP columns that were specifically developed for the analysis of cationic water-soluble polymers.

Table 7 Types of water-soluble polymers

<table>
<tr><th colspan="6">Water-soluble polymers</th></tr>
<tr><th colspan="2">Natural polymers</th><th colspan="2">Semi-synthetic polymers</th><th>Synthetic polymers</th></tr>
<tr><td>Starches</td><td>Sweet potato starch
Potato starch
Wheat starch</td><td rowspan="2">Cellulosics</td><td rowspan="2">Viscose
Methylcellulose
Ethylcellulose
Hydroxyethyl cellulose
Carboxymethyl cellulose</td><td rowspan="2">Polyvinyl alcohol
Polyethylene oxide
Polyvinyl ether
Polyvinylpyrrolidone
Polyacrylamide</td></tr>
<tr><td>Mannan</td><td>Konnyaku</td></tr>
<tr><td>Seaweed</td><td>Glue plant (funori)
Agar (galactan)
Sodium alginate</td><td>Starches</td><td>Soluble starch
Carboxymethyl starch
Dialdehyde starch</td><td rowspan="2">Sodium polyacrylate
Sodium polystyrene sulfonate
Polyacrylamine
Polyvinylpyridine hydrochloride
Polyethylenimine</td></tr>
<tr><td>Plant mucilage</td><td>Abelmosk
Tragacanth gum,
Gum arabic</td><td rowspan="3"></td><td rowspan="3"></td></tr>
<tr><td>Viscous substances derived from microbials</td><td>Dextran
Levan</td><td rowspan="2"></td></tr>
<tr><td>Proteins</td><td>Glue
Gelatin
Casein
Collagen</td></tr>
</table>

Table 8 Types of Eluents

Type	Representative Eluent for use with TSKgel PW columns
Non-electrolyte polymers and polyanions	0.2mol/L phosphate buffer, pH 7, or 20% CH_3CN (or CH_3OH) / 0.2mol/L phosphate buffer, pH 7
Polycations	0.5mol/L acetate + 0.5mol/L sodium acetate or 0.3mol/L TEA + conc. phosphoric acid, pH 2.9

TEA: Triethanolamine

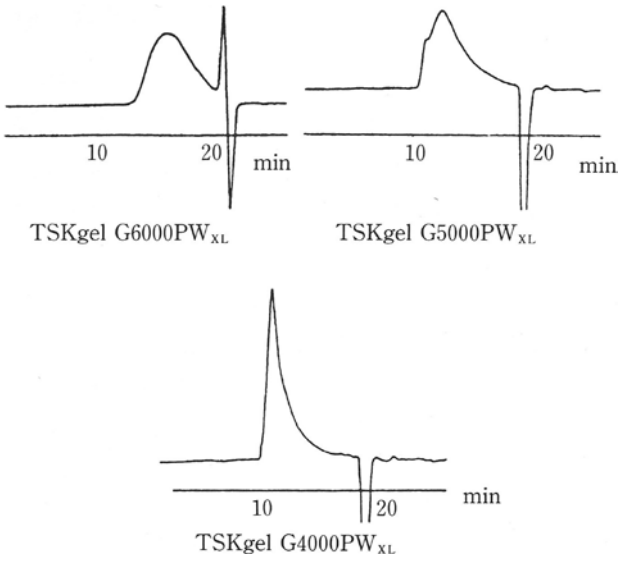


Figure 18 Separation of sodium polyacrylate

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.2mol/L phosphate buffer, pH 6.9

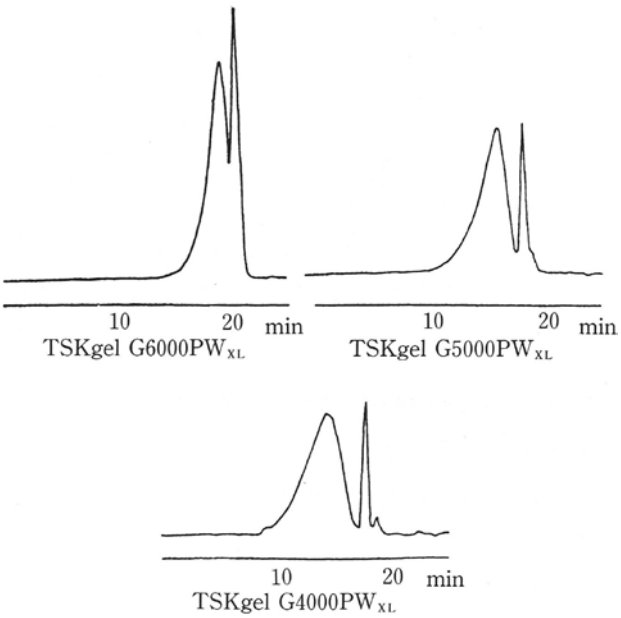
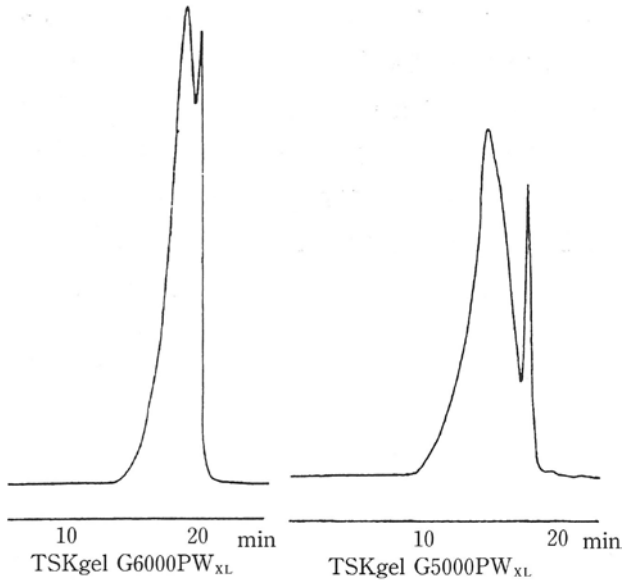


Figure 19 Separation of DNA sodium salt

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.2mol/L phosphate buffer, pH 6.9



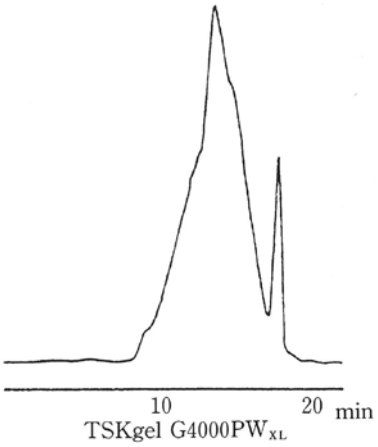


Figure 20 Separation of gelatin

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.2mol/L phosphate buffer, pH 6.9

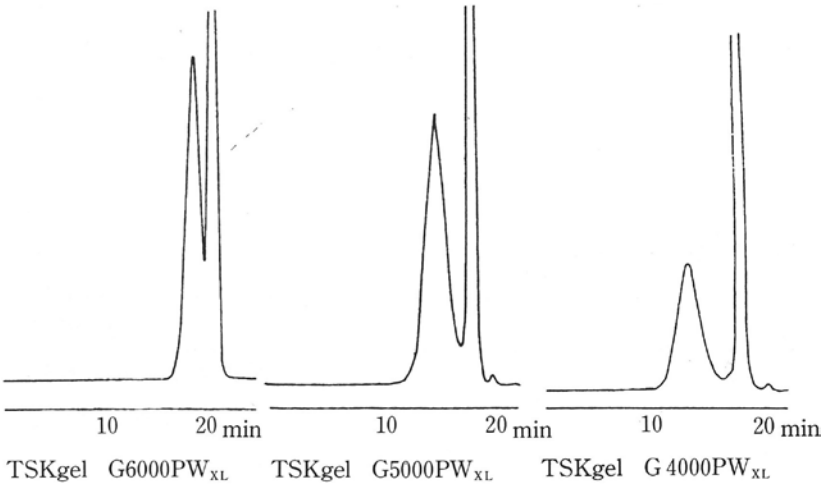


Figure 21 Separation of chondroitin sulfate

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.2mol/L phosphate buffer, pH 6.9

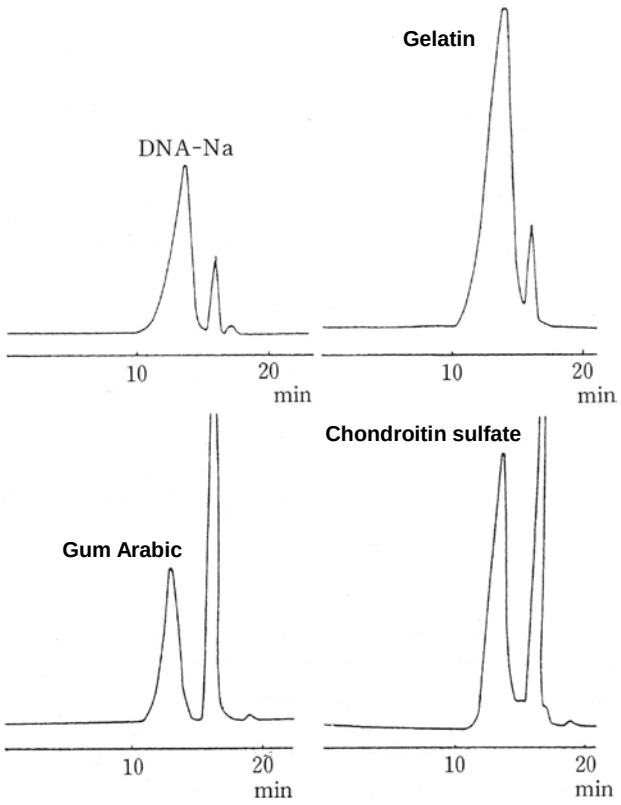


Figure 22 Separation performed using TSKgel GMPW$_{XL}$ column (1)

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.2mol/L phosphate buffer, pH 6.9

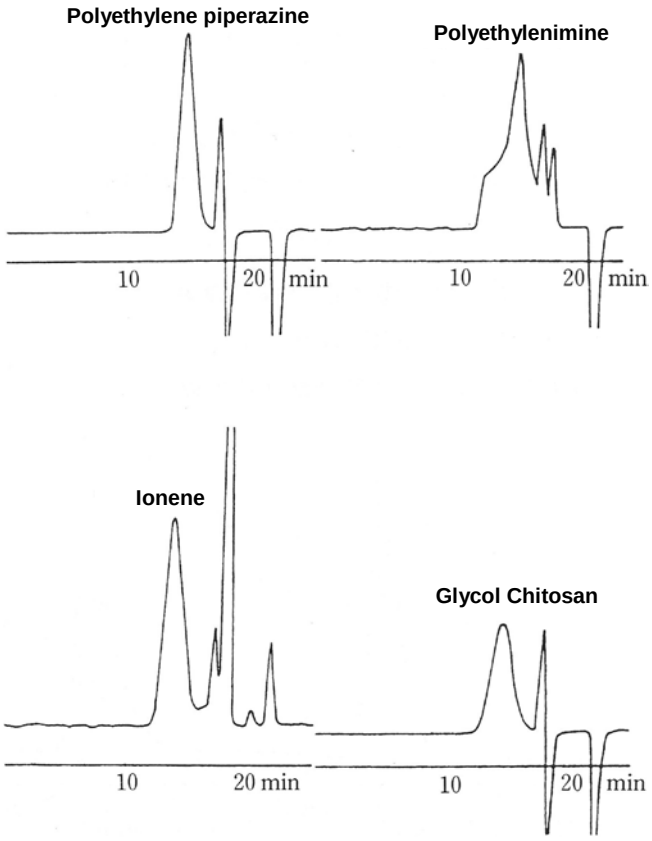


Figure 23 Separation performed using TSKgel GMPW$_{XL}$ column (2)

Column size: 7.8mm ID × 30cm × 2
Eluent: 0.5mol/L phosphate buffer + 0.5mol/L sodium acetate

10. Conclusions

When used for *analytical* purposes, TSKgel PW_{XL} columns provide improved performance over conventional GFC columns. In addition to an improvement in overall separation efficiency, 30cm high performance TSKgel PW_{XL} columns offer several advantages over 60cm TSKgel PW columns, including

- A two-fold reduction in analysis time and solvent use
- Increased peak height and thus higher sensitivity

.

Note: While 30cm TSKgel PW_{XL} columns are preferred over 60cm TSKgel PW columns for *analytical* separations, for *preparative* purposes 60cm TSKgel PW columns outperform the shorter TSKgel PW_{XL} columns simply because they contain more packing material, which allows a proportional increase in sample mass that can be injected.

For further information about the TSKgel PW_{XL} and PW columns, we recommend that you consult the following Separation Reports available at www.tosohbioscience.com:

SR037: Introduction of Aqueous SEC Columns: TSKgel PW_{XL} series

SR106: Aqueous SEC Columns for Analysis of Cationic Polymers: TSKgel PW_{XL}-CP Series