TOSOH

No.038

SEPARATION REPORT

GFC separation of water-soluble polymers with TSKgel PW-type columns (2)
— using the PWXL series —

— Contents —

1. Introduction

During the last dozen years, high-performance GPC and GFC have gained widespread use due to their ability to facilitate separation, good reproducibility, and short separation time.

As shown in Table 1, which indicates the years in which various packing materials for GPC and GFC were developed, Tosoh has led the world in developing high-performance GPC and GFC columns for both organic solvents and aqueous solvent systems.

Now, at a time when demand is rising for faster, more sensitive, and labor-saving analytical equipment, Tosoh is developing high-performance high resolution columns for both organic solvent systems and aqueous solvent systems.

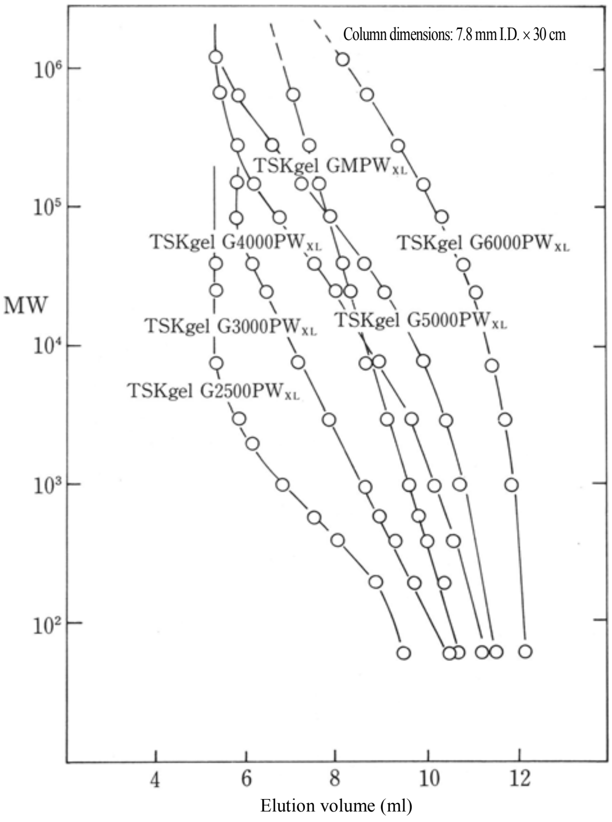
This report discusses the separation performance of the high-performance PWXL series of GPC columns for use in aqueous systems, and presents applications for using the PWXL series.

Table 1 History of development of GPC and GFC packing materials

		Historical background
1970	Development of S- and H-type GPC packing materials for organic solvent systems	Petrochemical industry is flourishing
1975	Development of SW- and PW-type GFC packing materials for aqueous solvent systems	Oil shock Growth of high value-added industries
1980	Development of TOYOPEARL packing materials for preparative GFC Development of packing materials for GPC and GFC with high theoretical plate numbers	Analytical equipment becoming faster, more sensitive, and more laborsaving

2. Calibration curves, theoretical plate numbers and separation range for each grade in the PWXL series

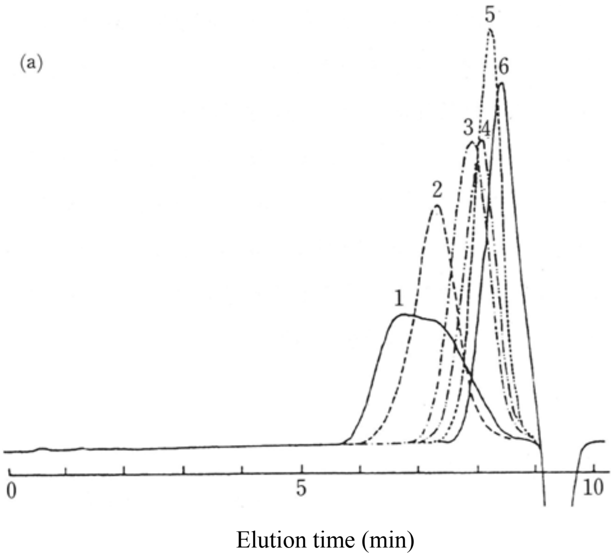
Figure 1 shows calibration curves for each column in the PWXL series using a standard polyethylene oxide (PEO) SE series sold by Tosoh. TSKgel GMPWXL has been added which is absent from the conventional PW series of columns. The TSKgel GMPWXL is a series of columns designed so that when several grades of columns with different pore sizes are combined, the calibration curve will become linear over a wide range of molecular masses, making this system suitable for analyzing molecular mass and molecular mass distributions. The TSKgel G2500PWXL has also been added. In this grade, ion specificity is vastly improved over that of the conventional TSKgel G2000PW column, while the resolution is maintained (for details see Separation Report No. 37).



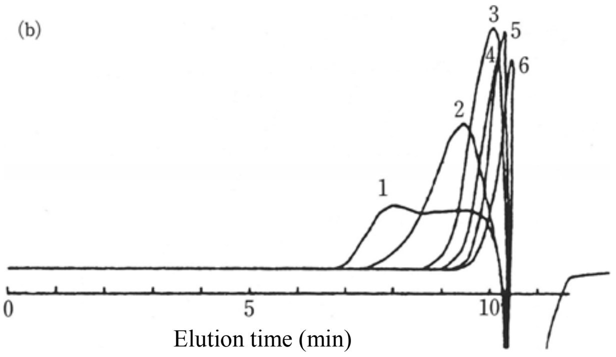
Sample: Standard polyethylene oxide

Figure 1 Calibration curves for the PWXL series

Table 2 shows the name, particle size, theoretical plate number and molecular mass exclusion limit of each grade. Figures 2a through 2d are chromatograms produced by separating dextran using the TSKgel GMPWXL, TSKgel G6000PWXL, TSKgel G5000PWXL, and TSKgel G4000PWXL. Consult Figures 1 and 2 and Table 2 to choose the optimum column system for the sample to be analyzed.



Column: TSKgel GMPWXL, 7.8 mm I.D. × 30 cm



Column: TSKgel G6000PWXL, 7.8 mm I.D. × 30 cm

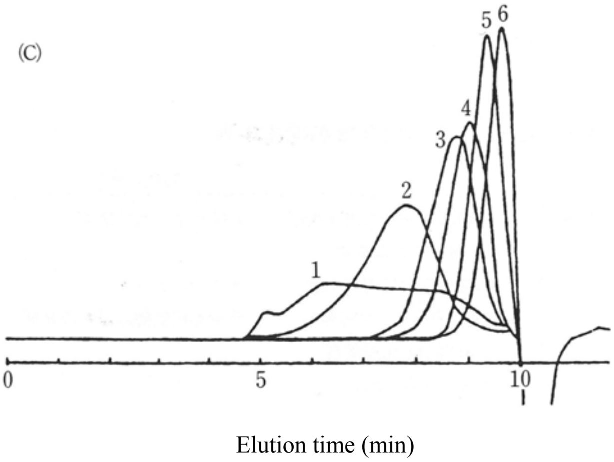
Figure 2 Separation of dextran

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

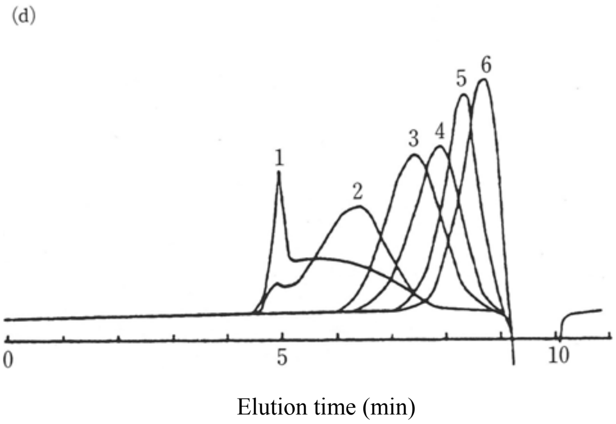
Table 2 TSKgel PWXL Series

Name	Particle size	Theoretical plate number	Exclusion limit
G2500PWXL	6µm	14000TP	10,000Da
G3000PWXL	6	14000	100,000
G4000PWXL	10	10000	1,000,000
G5000PWXL	10	10000	4,000,000
G6000PWXL	13	7000	—
GMPWXL	13	7000	—
G-Oligo-PW	6	14000	10,000
G-DNA-PW	10	—	—

(Size of each column: 7.8 mm I.D. × 30 cm)



Column: TSKgel G5000PWXL, 7.8 mm I.D. × 30 cm



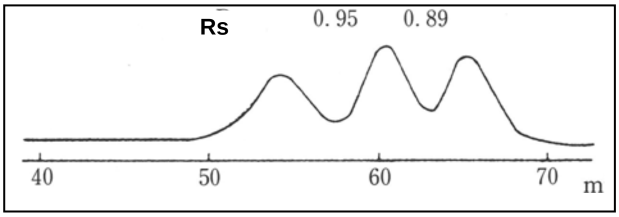
Column: TSKgel G4000PWXL, 7.8 mm I.D. × 30 cm

3. Comparison of performance of PW$_{XL}$ series and PW series

Figures 3 and 4 are chromatograms comparing the performance of the PW and PW$_{XL}$ series of columns using a TSKgel G6000PW$_{(XL)}$ + TSKgel G3000PW$_{(XL)}$ system of columns.

Figure 3 compares elution profiles and resolution (Rs) produced using a mixture of polyethylene oxide standards (SE 150: Mw = 1.20 $\times 10^6$; SE 30: Mw = 2.8 $\times 10^6$; SE 8: Mw = 7.3 $\times 10^4$) as the sample. Figure 4 compares elution profiles produced using a mixture of pullulan standards (P-400: Mw = 33.8 $\times 10^4$; P-50: Mw = 4.67 $\times 10^4$) as the sample. As columns in the PW$_{XL}$ series are half as long as the 60-cm columns in the PW series, separation is completed in half the time, while resolution shows equivalent or higher resolution than results produced with the PW series.

PW Series
Column size: 7.5 mm I.D. × 60 cm × 2



PW$_{XL}$ Series
Column size: 7.8 mm I.D. × 30 cm × 2

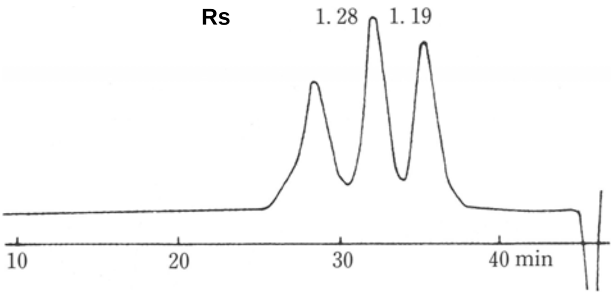
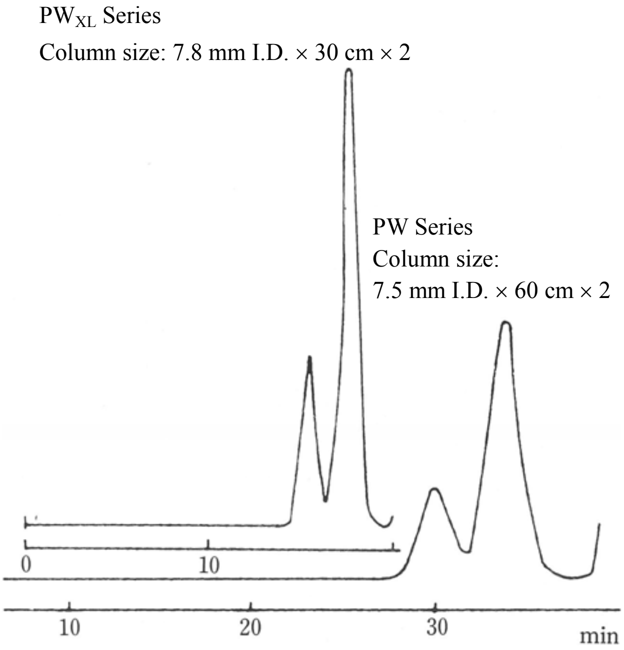


Figure 3 Comparison of PW and PW$_{XL}$ Series (1)

Sample: Polyethylene oxide, SE150-SE30-SE8
Injection volume: 100 µL (0.4 g/L)
Column: TSKgel G6000 PW + TSKgel G3000 PW
Temperature: 50 °C
Eluent: 0.1 mol/L NaCl
Flow rate: 0.5 mL/min


PW$_{XL}$ Series
Column size: 7.8 mm I.D. × 30 cm × 2

PW Series
Column size:
7.5 mm I.D. × 60 cm × 2

Figure 4 Comparison of PW and PW$_{XL}$ Series (2)

Sample: Pullulan, P400-P50
Injection volume: 100 µL (0.4, 1.2 g/L)
Column: TSKgel G6000 PW + TSKgel G3000 PW
Temperature: 50 °C
Eluent: 0.1 mol/L NaCl
Flow rate: 1.0 mL/min

Figure 5 shows the results of a comparison of the performance of PWXL series with the PW series in a system using a TSKgel G4000 PW column by itself. A mixture of polyethylene oxide standards (SE 150: Mw = 1.20 × 10^6; SE 15: Mw = 15 × 10^4; SE 2: Mw = 2.5 × 10^4) was used as the sample. Results were similar to those shown in Figures 3 and 4 were obtained.

4. Effect of elution conditions on resolution of the PWXL series

1) Effect of flow rate

Figure 6 compares elution profiles at flow rates of 1.0 mL/min and 0.5 mL/min. Resolution (Rs) between the peaks are noted on the chromatographs. Results show that resolution improves as the flow rate decreases.

2) Effect of temperature

Figure 7 shows the results of a comparison of the elution profiles occurring when columns were placed in a column oven at 25 °C and 50 °C, at a constant flow rate (0.5 mL/min). Increasing the temperature causes the resolution between the peaks to increase significantly.

3) Effect of sample concentration

Figure 8 compares elution profiles produced when the sample concentration of each component was 0.4 g/L versus 1.6 g/L. Elution profiles are very sensitive to the sample concentration and change dramatically as shown in Figure 8. In general, separation improves as the concentration of the sample decreases.

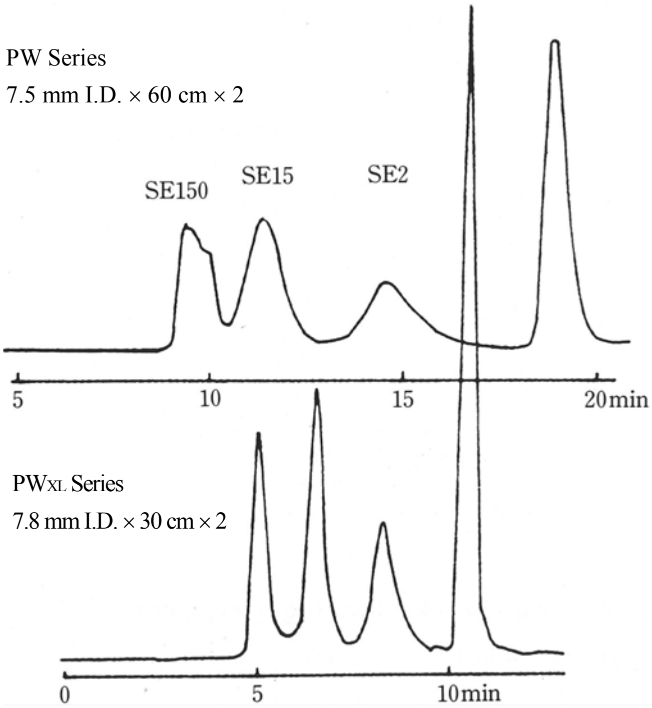


Figure 5 Comparison of PW and PWXL Series (3)

Sample: Standard polyethylene oxide
Injection volume: 100 µL
Column: TSKgel G4000 PW
Temperature: 50 °C
Eluent: 0.1 mol/L NaCl
Flow rate: 1.0 mL/min

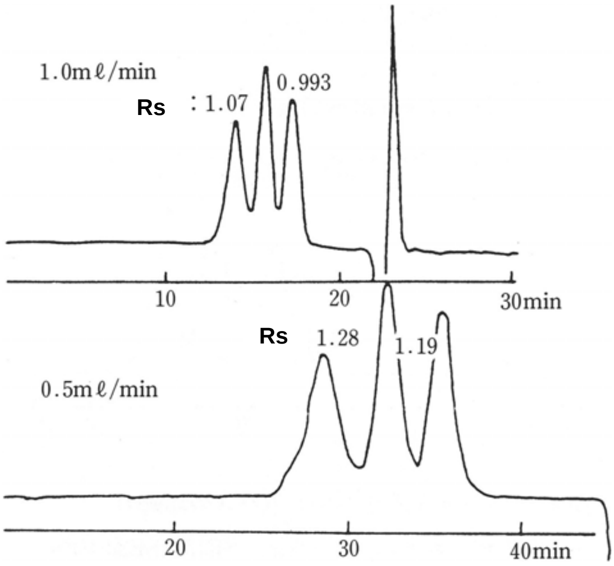


Figure 6 Effect of flow rate

Sample: Standard polyethylene oxide, SE150-SE30-SE8
Injection volume: 100 µL (0.4 g/L)
Columns: TSKgel G6000PWXL + TSKgel 3000 PWXL
7.8 mm I.D. × 30 cm × 2
Temperature: 50 °C
Eluent: 0.1 mol/L NaCl

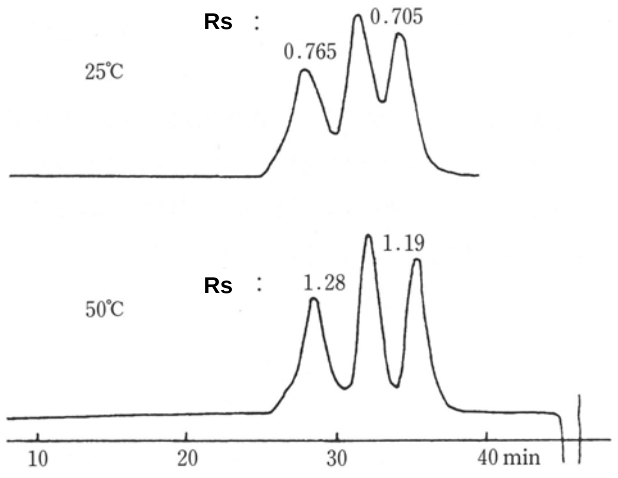


Figure 7 Effect of temperature

Sample: Standard polyethylene oxide,
SE150-SE30-SE8
Injection volume: 100 µL (0.4 g/L)
Columns: TSKgel G6000PWXL + TSKgel G3000PWXL
7.8 mm I.D. × 30 cm × 2
Eluent: 0.1 mol/L NaCl
Flow rate: 0.5 mL/min

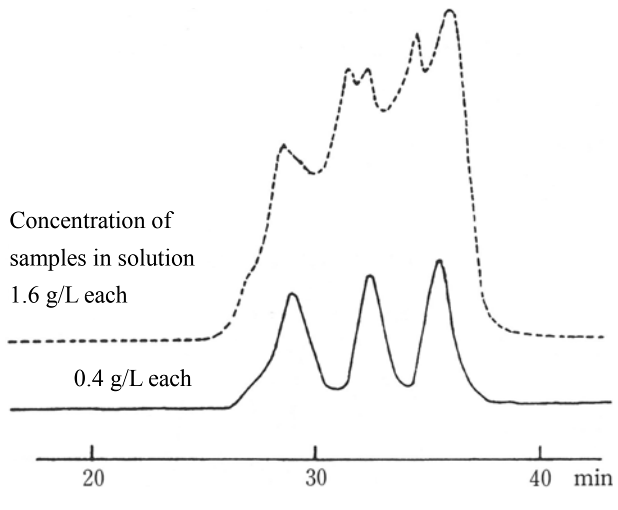


Figure 8 Effect of sample concentration in solution

Sample: Standard polyethylene oxide,
SE150-SE30-SE8
Injection volume: 100 µL (0.4 g/L)
Columns: TSKgel G6000PWXL + TSKgel G3000PWXL
7.8 mm I.D. × 30 cm × 2
Temperature: 50°C
Eluent: 0.1 mol/L NaCl
Flow rate: 0.5 mL/min

4) Effect of sample injection volume

Figure 9 compares the elution profiles produced with sample injection volumes of 100 µL and 500 µL. 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 PW series. The PWXL series is slightly more sensitive to injection volume than the PW series.

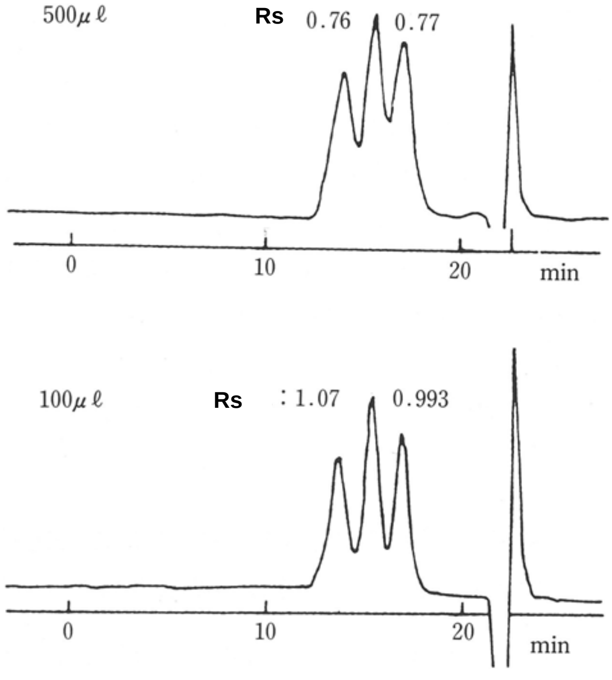


Figure 9 Effect of sample injection volume (1)

Sample: Standard polyethylene oxide,
SE150-SE30-SE8
Sample concentration: 0.4 g/L each
Columns: TSKgel G6000PWXL + TSKgel G3000PWXL
7.8 mm I.D. × 30 cm × 2
Temperature: 50°C
Eluent: 0.1 mol/L NaCl
Flow rate: 1.0 mL/min

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

In high-performance liquid chromatography, the separation between samples is expressed by Equation (1).

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

Here, Rs represents the resolution of a sample, with better separation indicated by larger values. N expresses the theoretical plate number; α as the separation factor of the sample and K′ as the capacity ratio are calculated using Equations (2) and (3), respectively.

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

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

(See Figure 11 for V_R and V_O)
The method for calculating the resolution Rs is shown in Figure 11.
In GPC, because the interaction between the sample and the packing material is not used, α and K′ are constants that are unrelated to the length of the column.
Thus Equation (1) becomes:

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

A simple way to increase the number of theoretical plates is to lengthen the column.

Figure 12 shows changes in the elution profiles for a mixed solution of standard polyethylene oxides produced when the length of the column is changed. Separation increases as the length of the column increases. Table 3 shows theoretical plate numbers N for SE15 at various column lengths as well as calculations of the Rs between peaks SE15 and SE2. R and $\sqrt{N}$ values are plotted in Figure 13. A very good linear relationship is achieved, satisfying the relationship expressed by equation (1′).

6. Dependence of Mw/Mn and Mz/Mw on theoretical plate number and sample concentration in GPC

Using standard polyethylene oxide SE15, pullulan P50 and P400, and dextran T70 as samples, changes in M_w/M_n and M_z/M_w values at varying column lengths were investigated using TSKgel G4000PWXL in the column. The results are shown in Figure 14. Values for dextran, pullulan and polyethylene oxide tended to near constant a level at a column length of ≥120 cm.

Figure 15 shows changes in Mw/Mn and Mz/Mw values produced when the concentration of the sample in solution was varied at a fixed column length of 30 cm. Mw/Mn and Mz/Mw values became constant in all solutions at a concentration range of 0.05-0.2% (injection volume: 100 μL).

For each of these samples, M_w/M_n and M_z/M_w values were not as sensitive to resolution as the theoretical plate number or resolution. However, to obtain accurate Mw/Mn and Mz/Mw values, a column length of around 120 cm is necessary when using TSKgel G4000PWXL.

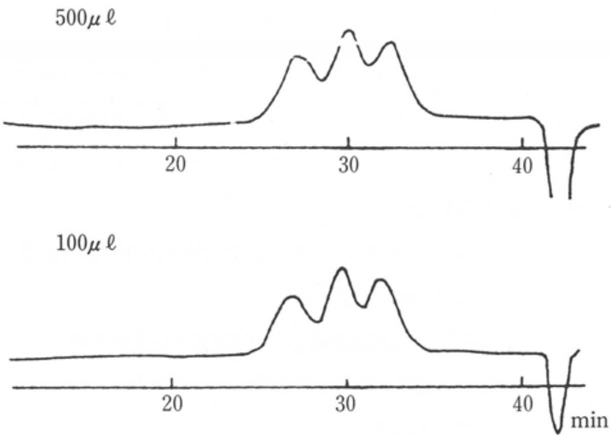


Figure 10 Effect of sample injection volume (2)

Sample:	Standard polyethylene oxide, SE150-SE30-SE8
Sample solution concentration: 0.4 g/L each	
Columns:	TSKgel G6000PW + TSKgel G3000PW 7.5 mm I.D. × 60 cm × 2
Temperature:	50°C
Eluent:	0.1 mol/L NaCl
Flow rate:	1.0 mL/min

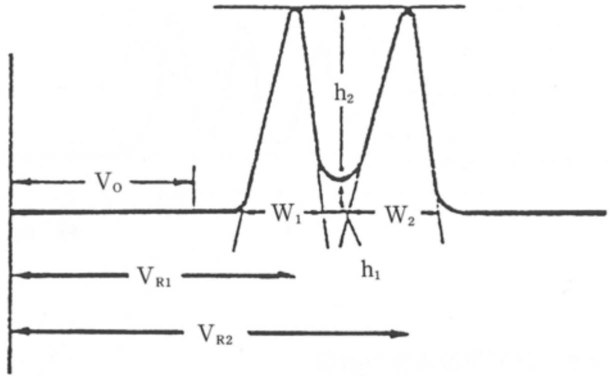


Figure 11 Method for calculating resolution factor

$$R = \frac{2\ (V_{R2}-V_{R1})}{W_1+W_2} \quad \cdots\cdots(a) \text{ or}$$

$$R = \frac{h_2}{h_1+h_2}\times 100 \quad \cdots\cdots(b)$$

In (a), the value increases as separation improves, while the value in (b) will become 100% when separation is complete.

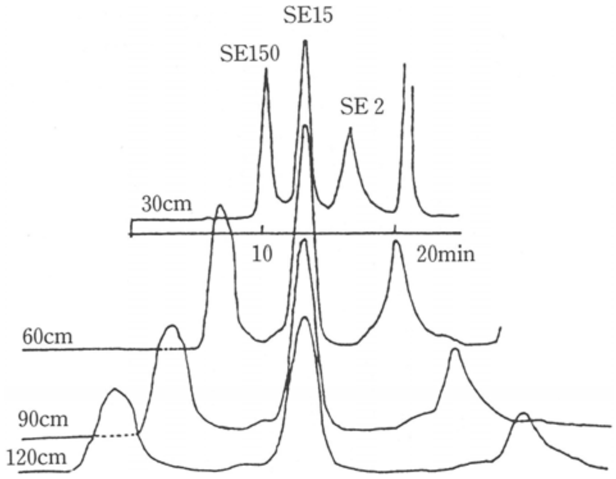


Figure 12 Dependence of changes in separation on column length

Sample:	Standard polyethylene oxide, SE150-SE15-SE2
Injection volume:	100 µL (0.4 g/L each)
Column:	TSKgel G4000PWXL 7.8 mm I.D.
Temperature:	50°C
Eluent:	0.1 mol/L NaCl
Flow rate:	0.5 mL/min

Table 3 Theoretical plate number and resolution factor (1)

Column length (cm)	N*	$\sqrt{N}$	R**
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 SE15.

**R: Resolution factor between peaks for SE15 and SE2.

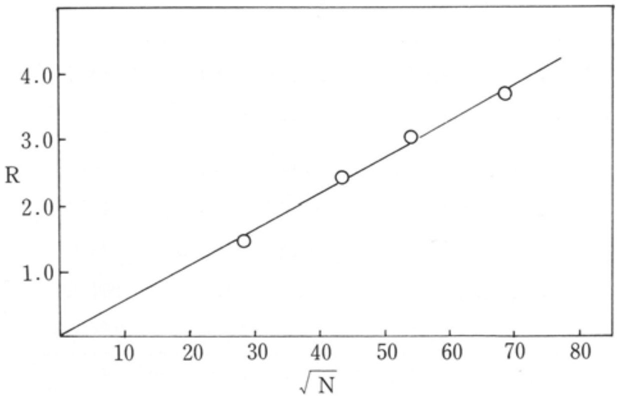


Figure 13 Theoretical plate number and resolution (2)

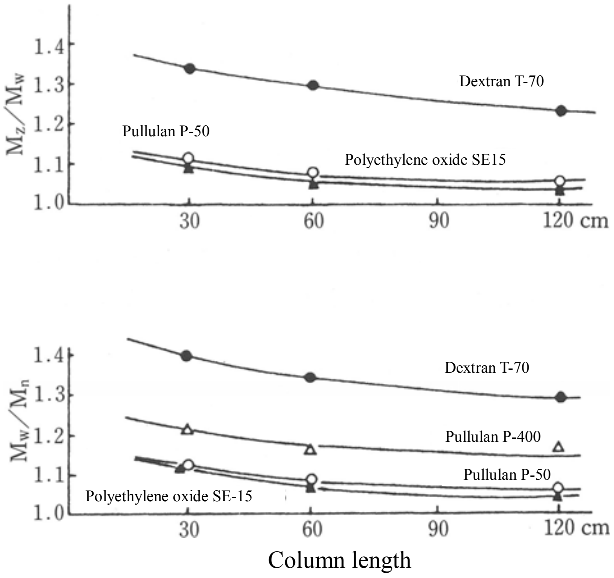


Figure 14 Dependence on theoretical plate number

Column:	TSKgel G4000PWXL 7.8 mm I.D.
Temperature:	50°C
Eluent:	0.1 mol/L NaCl
Flow rate:	1.0 mL/min
Injection volume:	100 µL (1.0 g/L each)

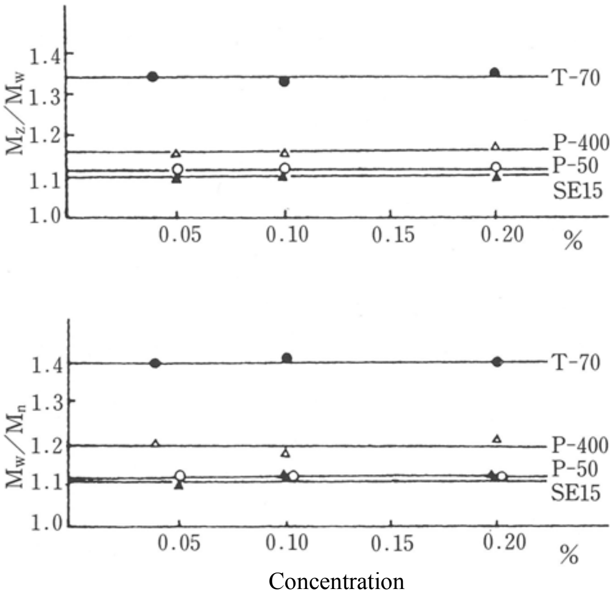


Figure 15 Dependence on concentration of sample solution

Column:	TSKgel G4000PWXL 7.8 mm I.D. × 30 cm
Temperature:	50 °C
Eluent:	0.1 mol/L NaCl
Flow rate:	1.0 mL/min
Injection volume:	100 µL

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

7. Analyzing molecular mass by GPC/LALLS (low-angle laser light scattering detector) using TSKgel GMPWXL

1) Analyzing weight-average molecular mass Mw of pullulan standard sample

Pullulan standard samples with a narrow molecular mass distribution are commercially available. Accordingly, the molecular mass of pullulan was subjected to GPC/LALLS separation using a TSKgel GMPWXL column. Figure 16 shows the GPC/LALLS chromatogram produced.

Table 4 shows the results of separation for each grade of pullulan. Human serum albumin (M=66,000) was used as the primary standard, with pullulan P100 as the secondary standard. For pullulan, molecular masses were determined by Professor Kawahara et al., by sedimentation equilibrium. Molecular masses were also determined by Professor Takagi et al., by GPC/LALLS, and a comparison found these results were very consistent.

2) Measuring the weight-average molecular mass Mw of standard sodium polystyrene sulfonate (PNaSS)

Standard sodium polystyrene sulfonate is commercially available from the American company, Pressure Chemical, as a standard polymer electrolyte with a narrow molecular mass distribution.

GPC separation of standard sodium polystyrene sulfonate requires the addition of at least 10 % acetonitrile or methanol to the eluent. Figure 18 shows chromatograms produced when standard sodium polystyrene sulfonate was analyzed using TSKgel GMPXL. Peak shapes on a chromatogram of standard sodium polystyrene sulfonate produced with 10 % added acetonitrile were essentially the same as when 20 % acetonitrile was added.

Table 5 shows the results of a study of the differences in the apparent molecular mass Mw of each sample with varying Eluent compositions. Small changes in the concentration of inorganic salt in the solvent or in the composition of an organic solvent caused only very slight changes in apparent Mw. However, the values resulting from analyses in each case were slightly higher than the nominal value provided by Pressure Chemical. Various explanations for this have been considered, including the complexity of the solvent composition, the inability of this method to handle light scattering in two-component solution, and the hygroscopic properties of the samples. This problem will be a subject of future research in GPC/LALLS.

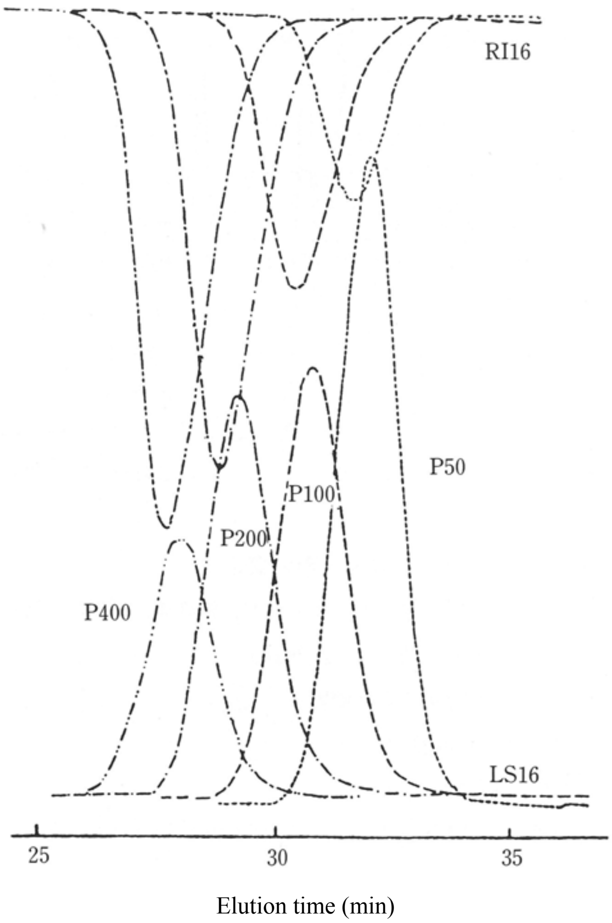


Figure 16 Chromatogram of pullulan by GPC/LALLS

Sample:	Pullulan
Injection volume:	500 µL
Column:	TSKgel GMPWXL 7.8 mm I.D. × 120 cm
Temperature:	40 °C
Eluent:	0.1 mol/L NaCl
Flow rate:	1.0 mL/min

Table 4 Results of pullulan molecular mass analysis

Grade of pullulan	M_w	Rf.1	Rf.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

Rf.1 Data from Professor Takagi et al.

Rf.2 Data from sedimentation equilibrium by Professor Kawahara et al.

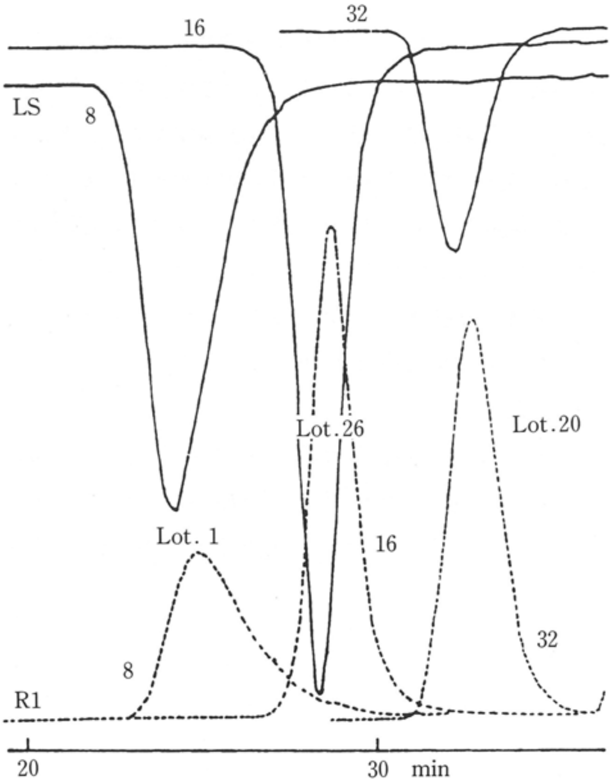


Figure 18 Separation of standard sodium polystyrene sulfonate

Sample: Standard sodium polystyrene sulfonate
Injection volume: 500 µL
Column: TSKgel GMPWXL
7.8 mm I.D. × 30 cm × 4
Temperature: 40 °C
Eluent: 10 % acetonitrile/0.2 mol/L Na_2SO_4
Flow rate: 1.0 mL/min

8. Dependence of Mn, Mw, and Mz on theoretical plate number with GPC/LALLS

Table 6 shows changes in Mn, Mw, and Mz when analysis was performed by GPC/LALLS at varying TSKgel GMPWXL column lengths, using dextran T500 as the sample. Values in parentheses indicate values without performing function approximation. (The necessity and validity of functional approximation is published in a separate report). Although slight, Mn, Mw, and Mz values do depend on the theoretical plate number. (As in normal GPC, a column with a certain capacity for high-resolution separation is necessary to evaluate Mn and Mz).

Table 6 Analysis of weight-average molecular mass by GPC/LALLS

Column length (cm)	$M_n\times10^{-4}$	$M_w\times10^{-4}$	$M_z\times10^{-4}$
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.

Sample: Dextran T500
Column: TSKgel GMPWXL
Solution: 0.1 mol/L NaCl

Table 5 Analysis of weight-average molecular mass of standard sodium polystyrene sulfonate by GPC/LALLS

	Apparent M_w			
Lot. No.	Solvent a	b	c	Nominal value
#1	1210000	1210000	1290000	1060000
#16	794000	787000	821000	690000
#26	202500	202000	210000	177000
#20	19400	21000		16000

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

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

Table 7 shows a table listing various types of representative water-soluble polymers. Although various types of water-soluble polymers exist, most samples can be analyzed by preparing several solvents for GPC use as shown in Table 8. However, analyzing viscose is not currently easy, as this substance can only be dissolved in a highly alkaline aqueous solution, or polymers that contain several aromatic rings (in monomer units).

To date, the separation of polycations has been considered to be difficult due to the fact that trace quantities of carboxyl groups are present in the gel. However, this problem can be solved by making the solvent acidic, inhibiting carboxyl group disassociation, and increasing the ionic strength. One problem is that, in our experience, if polyanions are analyzed after polycation separation has been performed, polyanions, which should be easy to analyze, show signs of adsorption. As this can cause various problems in separation, setting aside columns that are used only for polycation separation is preferable if possible. Several GPC applications of water-soluble polymers are shown in Figures 19-24.

Table 7 Types of water-soluble polymers

Water-soluble polymers				
Natural polymers		Semisynthetic polymers		Synthetic polymers
Starch	Sweet potato starch Potato starch Wheat starch	Cellulose	Viscose Methylcellulose Ethylcellulose Hydroxyethyl cellulose	Polyvinyl alcohol Polyethylene oxide Polyvinyl ether Polyvinylpyrrolidone
Mannan	Konnyaku		Carboxymethyl cellulose	Polyacrylamide
Seaweed	Glue plant (funori) Agar (galactan) Sodium alginate	Starch	Soluble starch Carboxymethyl starch Dialdehyde starch	Sodium polyacrylate Sodium polystyrene sulfonate Polyacrylamine Polyvinylpyridine hydrochloride
Plant mucilage	Abelmosk Tragacanth gum, Gum arabic			Polyethylenimine
Viscous substances derived from microbials	Dextran Levan			
Proteins	Glue Gelatin Casein Collagen			

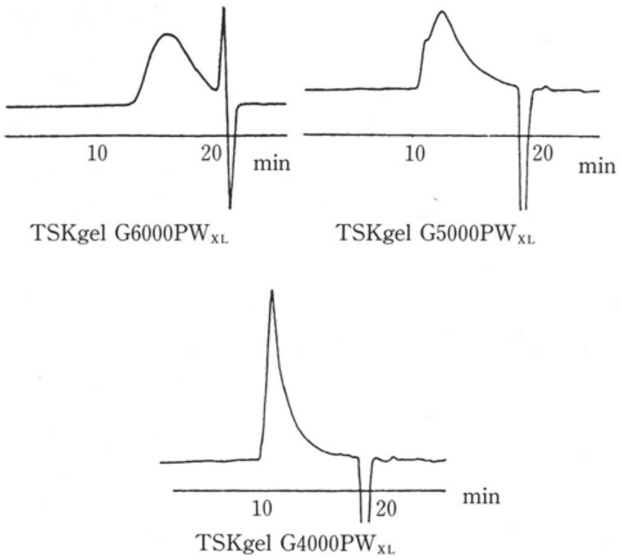


Figure 19 Separation of sodium polyacrylate

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.2 mol/L phosphate buffer (pH 6.9)

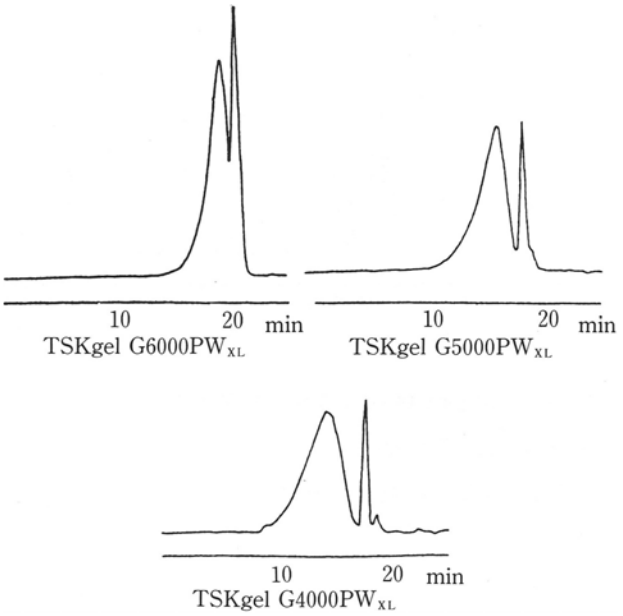


Figure 20 Separation of DNA-Na

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.2 mol/L phosphate buffer (pH 6.9)

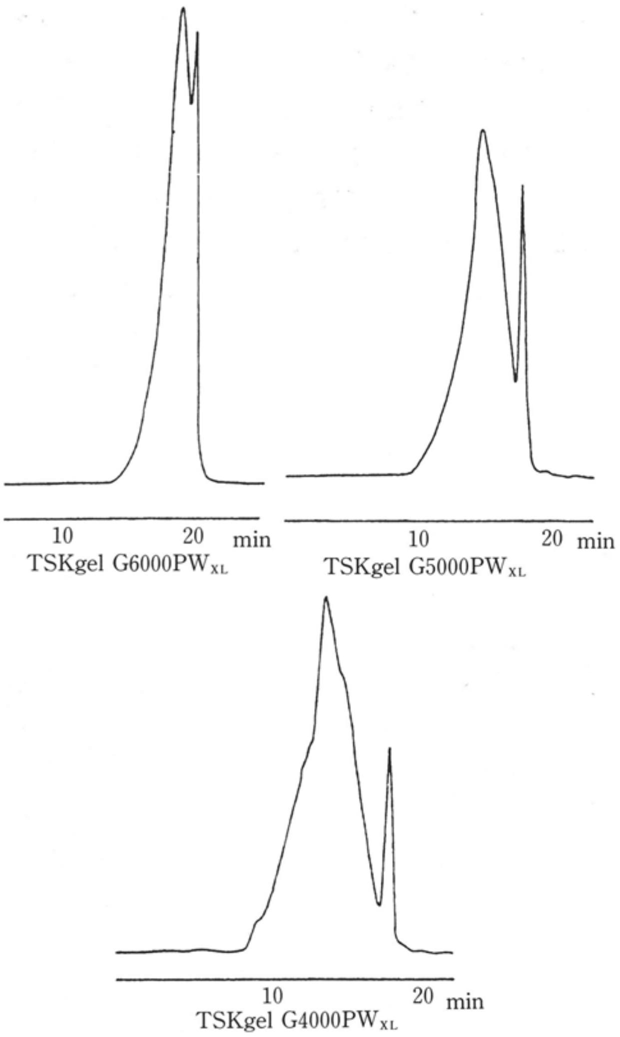


Figure 21 Separation of gelatin

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.2 mol/L phosphate buffer (pH 6.9)

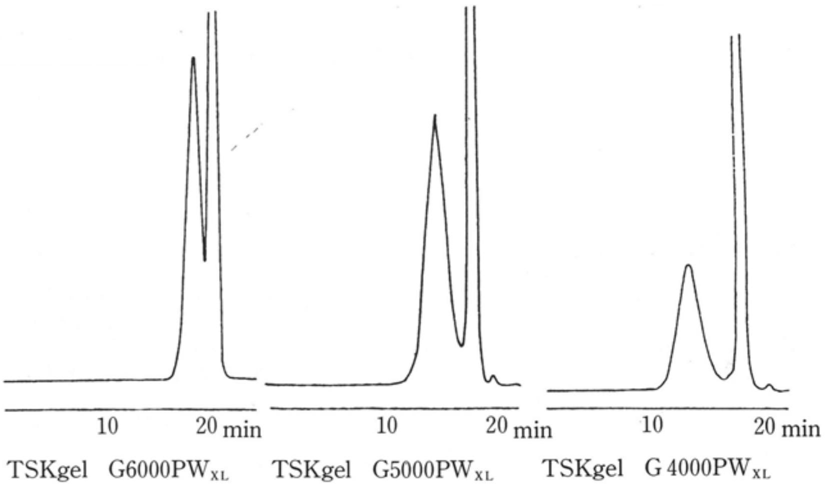


Figure 22 Separation of chondroitin sulfate

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.2 mol/L phosphate buffer (pH 6.9)

Figures 19-22 are chromatograms produced by analyzing the same samples using TSKgel G6000PWXL, TSKgel G5000PWXL, and TSKgel G4000PWXL, respectively. In Figure 23, the chromatogram shows the results of separation conducted using the TSKgel GMPWXL. Figure 24 shows the results of separation of polycations in an acetate buffer system using the TSKgel GMPWXL. Each of these chromatograms was produced at a flow rate of 1.0 mL/min with RI detection. See Separation Report No. 35 for further analytical details.

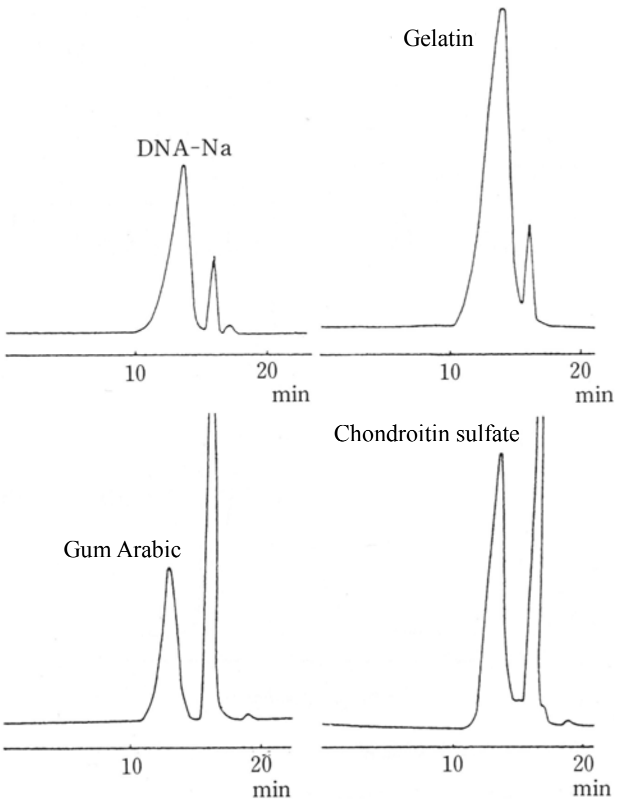


Figure 23 Separation performed using TSKgel GMPWXL (1)

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.2 mol/L phosphate buffer (pH 6.9)

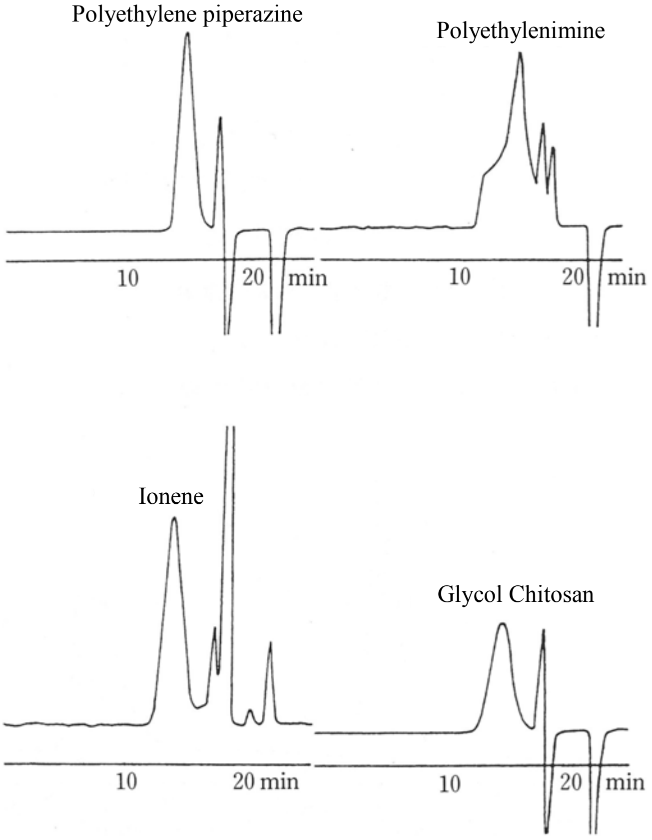


Figure 24 Separation Performed using TSKgel GMPWXL (2)

Column size: 7.8 mm I.D. × 30 cm × 2

Eluent: 0.5 mol/L phosphate buffer + 0.5 mol/L sodium acetate

Table 8 Types of Eluents

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

TEA: Triethanolamine

10. Conclusion

The TSKgel PW$_{XL}$ series of columns provides much improved performance over conventional GPC columns. Use of these columns offers many advantages: separation time is reduced by half while achieving high peak height and high sensitivity; and because the quantity of solvent used is reduced by half, separation efficiency is improved.

Given that high-performance GPC satisfies 3 essential elements (ease of use, speed, and high sensitivity), this method of separation is expected to see increasing use in the future.