No.106

SEPARATION REPORT

TOSOH

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

— Contents —

1. Introduction

Polymer and silica matrices are widely used as packing materials in aqueous size exclusion chromatography (SEC) for analyzing molecular mass distributions of water-soluble polymers. At Tosoh, we have developed and marketed the TSKgel PW and PW_{XL} series of aqueous SEC columns in which hydrophilic vinyl polymers are used as matrices.

When analyzing the molecular mass distribution of water-soluble polymers by SEC, it is generally necessary to optimize the type and concentration of the salt, as well as the pH of the eluent, in order to inhibit interaction between the sample and the packing material.

In particular, for SEC analyses of cationic polymers, to inhibit electrostatic interactions with the packing material, it is necessary to use low-pH eluents or eluents with high salt concentrations. However, even under these conditions, electrostatic interactions with the packing material may not be inhibited, and due to the resulting low sample recovery rates and poor repeatability, adequate molecular mass data is not always obtained.

To overcome these problems, we have developed and started to market the TSKgel PW_{XL}-CP series of aqueous SEC columns to enable analysis of cationic polymers with high recovery rates and good repeatability, even under mild conditions in which neutral eluents are used.

In this report the basic characteristics and application examples of the new TSKgel PW_{XL}-CP series of SEC columns will be discussed.

2. Features

When the TSKgel PW_{XL} series is used to analyze cationic polymers by SEC, adsorption may be observed, caused by electrostatic interactions between the sample and carboxyl groups present in trace amounts on the surface of the packing material. This can make it impossible for adequate molecular mass distribution data to be obtained.

The TSKgel PW_{XL}-CP series, developed using a new method of synthesis, uses the same matrix as the TSKgel PW_{XL} series. However, by improving the ionicity on the surface of the packing material, while maintaining the basic characteristics of the existing series of columns, this series provides excellent repeatability and high recovery rates for cationic polymers, even under conditions in which neutral eluents with low salt concentrations are used.

The basic characteristics of the TSKgel PW_{XL}-CP series are shown in **Tables 1** and **2**.

Table 1 Properties of the TSKgel PW_{XL}-CP series

	TSKgel G3000PW_{XL}-CP	TSKgel G5000PW_{XL}-CP	TSKgel G6000PW_{XL}-CP
Packing material matrix	Hydrophilic vinyl polymer	Hydrophilic vinyl polymer	Hydrophilic vinyl polymer
Particle size	7 μm	10 μm	13 μm
Molecular mass exclusion limit (PEO)	100,000	1,000,000	20,000,000*
Molecular mass separation range (PEO and PEG)	200～50,000	400～500,000	1,000～10,000,000

*Estimated

Table 2 Specifications of TSKgel PW_{XL}-CP series

Product name	Number of theoretical plates (TP/column)	Asymmetry factor	Column size (mm I.D. x cm)
TSKgel G3000PW_{XL}-CP	16,000/30 cm	0.7 ~ 1.6	7.8 x 30
TSKgel G5000PW_{XL}-CP	10,000/30 cm	0.7 ~ 1.6	7.8 x 30
TSKgel G6000PW_{XL}-CP	7,000/30 cm	0.7 ~ 1.6	7.8 x 30

Analysis conditions

Eluent: 0.1 mol/L aqueous solution of sodium nitrate
Detection: RI
Flow rate: 1.0 mL/min (7.8 mm I.D. x 30 cm)
Temperature: 25 ºC
Sample: Ethylene glycol (5 g/L)
Injection volume: 20 μL

3. Basic characteristics

3-1. Pore characteristics

The TSKgel PWXL-CP series, as shown in **Tables 1** and **2**, is provided in three grades with different molecular mass separation ranges capable of accommodating low- to high-molecular mass samples.

Figure 1 shows calibration curves produced with standard polyethylene oxide and polyethylene glycol in a 0.1 mL/L aqueous solution of sodium nitrate.

The TSKgel G3000PWXL-CP, the low-molecular mass grade column, is capable of analysis within a molecular mass separation range of 50,000 to 200. A molecular mass separation range of 500,000 to 400 was established for the TSKgel G5000PWXL-CP, and 10,000,000 to 1,000 for the TSKgel G6000PWXL-CP series, the high-molecular mass grade column. As a result, by connecting these columns together according to the molecular mass and molecular mass distribution of the sample, this series can accommodate a wide range of samples from high-molecular mass polymers to low-molecular mass oligomers.

3-2. Sample injection volume and height equivalent to theoretical plate (HETP)

Figure 2 shows the dependence of the height equivalent to theoretical plate (HETP) of ethylene glycol on injection volume in the TSKgel PWXL-CP series.

It is clear from this graph that at each grade, if the injection volume exceeds 50 μL when 2 columns are used, HETP begins to increase, and that in the TSKgel PWXL-CP series, the maximum sample injection volume is about 25 μL per column.

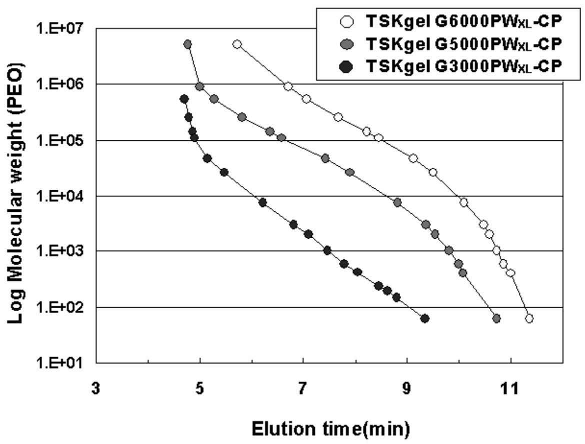

Fig. 1 TSKgel PWXL-CP series calibration curves

Column: TSKgel PWXL-CP series (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Standard polyethylene oxide, polyethylene glycol, and ethylene glycol
Injection volume: 100 μL

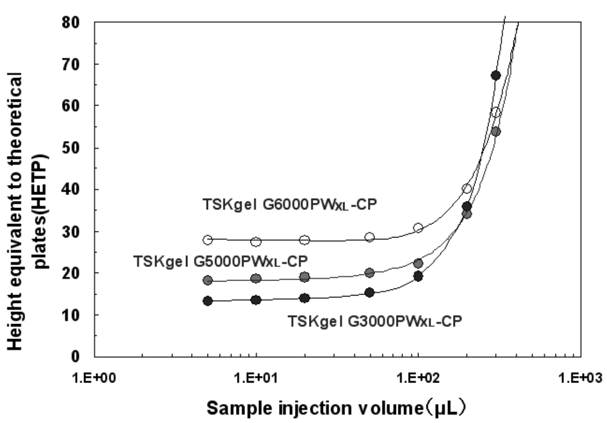

Fig. 2 Relationship between HETP and sample injection volume in TSKgel PWXL-CP series

Column: TSKgel PWXL-CP series (7.8 mm I.D. x 30 cm) x 2
Eluent: H_2O
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Ethylene glycol (5 g/L)
Injection volume: 5 to 500 μL

3-3. Dependence of height equivalent to theoretical plate (HETP) on flow rate

Figure 3 confirms the dependence of HETP on flow rate in the TSKgel PW_{XL}-CP series when EG, a low-molecular mass monodisperse material, is used as the sample.

The optimal flow rate (minimum HETP) varies in accordance with the particle size. The optimum flow rate (minimum HETP) with the TSKgel G3000PW_{XL}-CP, which has a small particle size, is relatively high (0.6 to 1.0 mL/min). However, as the particle size increases, the optimal flow rate decreases to 0.5 to 0.8 mL/min with the TSKgel G5000PW_{XL}-CP, and to 0.4 to 0.7 mL/min with the TSKgel G6000PW_{XL}-CP.

3-4. Separation performance

Although it was previously stated that with regard to pore characteristics, there are no major differences between the TSKgel PW_{XL}-CP series and TSKgel PW_{XL} series of columns, the pore characteristics of the TSKgel G3000PW_{XL}-CP, the low-molecular mass grade column, have been improved in comparison to the TSKgel G3000PW_{XL}.

Fig. 4 shows chromatograms of polyethylene glycol 200 (PEG 200) produced using the TSKgel G3000PW_{XL}-CP and the TSKgel G3000PW_{XL} columns.

The TSKgel G3000PW_{XL}-CP, which has improved pore characteristics in the low-molecular mass range, shows better separation than the TSKgel G3000PW_{XL}.

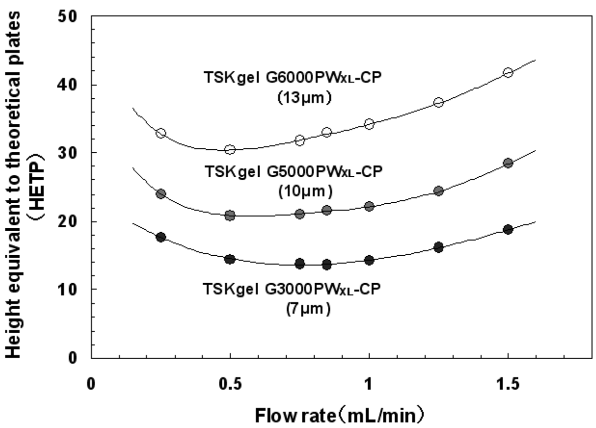

Fig. 3 Relationship between HETP and flow rate in the TSKgel PW_{XL}-CP series

Column: TSKgel PW_{XL}-CP series (7.8 mm I.D. x 30 cm)
Eluent: H_2O
Flow rate: 0.25 to 1.5 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Ethylene glycol (5 g/L)
Injection volume: 20 μL

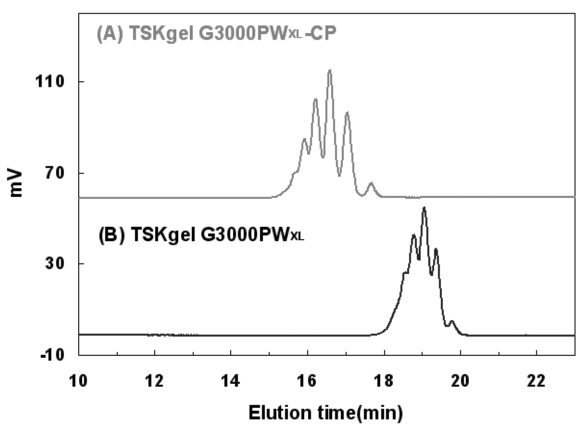

Fig. 4 Separation of Polyethylene Glycol 200 using the TSKgel G3000PW_{XL}-CP and TSKgel G3000PW_{XL}

Column: (A) TSKgel G3000PW_{XL}-CP (7.8 mm I.D. x 30 cm x 2)
(B) TSKgel G3000PW_{XL} (7.8 mm I.D. x 30 cm x 2)
Eluent: H_2O
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Polyethylene Glycol 200 (3 g/L)
Injection volume: 50 μL

4. Dependence of calibration curves on eluent

4-1. Temperature dependence

Fig. 5 shows the results of an investigation of the temperature-dependence of calibration curves with standard polyethylene oxide, using the TSKgel G5000PWXL-CP, with 0.1 mol/L aqueous solution of sodium nitrate as the eluent. The figure confirms that there is no temperature dependence in the range from 25 ºC to 60 ºC.

4-2. Dependence on pH in eluent

Fig. 6 shows calibration curves produced with standard polyethylene oxide when the pH of the eluent (0.1 mol/L acetate buffer) is varied, using the TSKgel G5000PWXL-CP.

The calibration curve showed no changes within a pH range of 4.5 to 8.3.

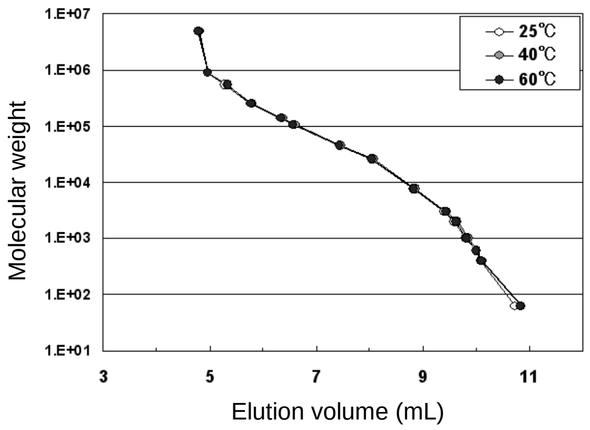

Fig. 5 Dependence of calibration curves on temperature in TSKgel G5000PWXL-CP

Column: TSKgel G5000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Temperature: 25 ºC, 40 ºC, 60 ºC
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Injection volume: 100 µL

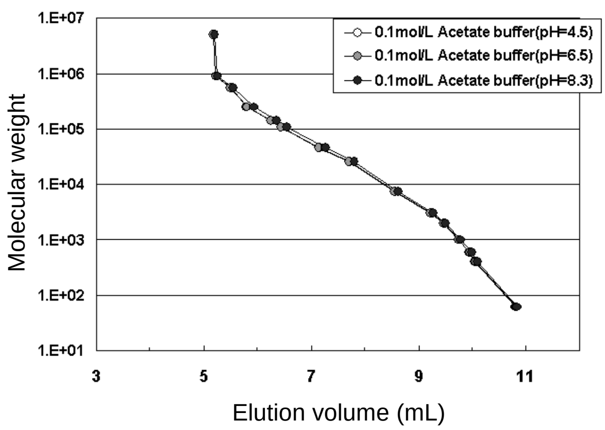

Fig. 6 Dependence of calibration curves on pH of eluent in TSKgel G5000PWXL-CP

Column: TSKgel G5000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L acetate buffer (pH = 4.5, 6.5, 8.3)
Flow rate: 1.0 mL/min
Temperature: 25 ºC
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Injection volume: 100 µL

4-3. Effects of organic solvent concentration in eluent

Fig. 7 shows the results of an investigation of the effect of acetonitrile concentration on the calibration curve using the TSKgel G5000PWXL-CP as the column, when acetonitrile is added to the eluent (0.1 mol/L sodium nitrate). In an acetonitrile concentration range of 0 to 20% very little impact on the calibration curve can be seen. As the acetonitrile concentration increases, elution of the standard samples becomes more rapid, and overall the calibration curve tends to shift toward the low side.

4-4. Flow rate dependency

Fig. 8 shows the results of an investigation of the dependency of standard polyethylene oxide calibration curves on the flow rate, using the TSKgel G6000PWXL-CP column and 0.1 mol/L sodium nitrate as the eluent. The results confirmed that there were no marked changes in the calibration curve over a range of flow rates from 0.25 to 1.0 mL/min.

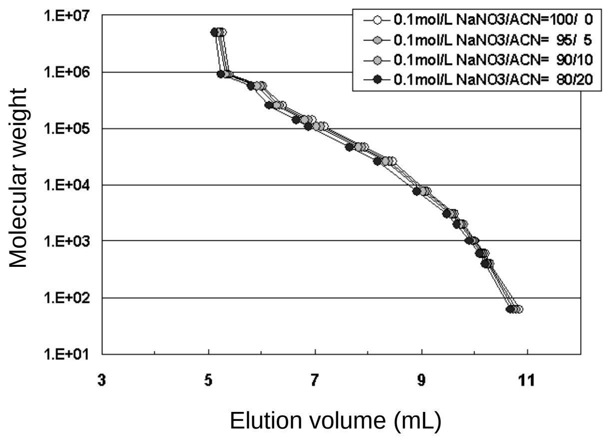

Fig. 7 Effect of concentration of organic solvent on calibration curve of TSKgel G5000PWXL-CP

Column: TSKgel G5000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate/acetonitrile (0/100 to 20/80)
Flow rate: 1.0 mL/min
Temperature: 25 ºC
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Injection volume: 100 μL

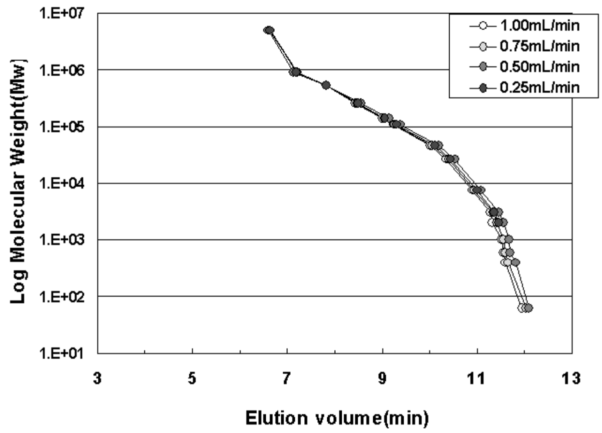

Fig. 8 Dependence of TSKgel G6000PWXL-CP calibration curves on flow rate

Column: TSKgel G6000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 0.25 to 1.0 mL/min
Temperature: 25 ºC
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Injection volume: 100 μL

5. Elution characteristics of cationic polymers

5-1. Comparison of elution using commercial SEC column

Fig. 9 shows the results of a comparison of the elution characteristics of poly(allylamine hydrochloride) (PAA-HCl), a cationic polymer, under mild solvent conditions [neutral solvents with low salt concentration (0.1 mol/L aqueous solution of sodium nitrate)] with the TSKgel G5000PW$_{XL}$-CP, TSKgel G5000PW$_{XL}$, and a commercial SEC column.

Due to adsorption, the cationic polymer PAA-HCl completely failed to elute in the PW$_{XL}$ and the commercial aqueous SEC columns. However, the chromatogram obtained with the TSKgel G5000PW$_{XL}$-CP series showed good elution of the sample.

Fig. 10 shows chromatograms obtained analyzing PAA-HCl with a multi-angle light scattering detector (MALS) using the TSKgel G6000PW$_{XL}$-CP and TSKgel G6000PW$_{XL}$, under the same solvent conditions as in **Fig. 9** (0.1 mol/L aqueous solution of sodium nitrate).

The figure verifies that even with MALS, PAA-HCl fails to elute in the TSKgel G6000PW$_{XL}$ due to adsorption and is not detected.

Generally, in aqueous SEC, eluents with high salt concentrations are used to inhibit electrostatic interaction with cationic polymers.

Fig. 11 shows a chromatogram produced by repeated analyses of PAA-HCl using a commercial aqueous SEC column in a eluent with a high salt concentration (0.5 mol/L acetate + 0.1 mol/L aqueous solution of sodium nitrate). The figure confirms that although a cationic polymer could be eluted under these solvent conditions, with a small number of injections the sample recovery rate was low, and repeatability was inadequate.

Fig. 12 shows the results of an investigation of the elution characteristics of PAA-HCl using each of the columns of the TSKgel PW$_{XL}$-CP series, in a 0.1 mol/L aqueous solution of sodium nitrate. Sample recovery rates and average molecular mass (Mw) data obtained by SEC analysis are also shown. The figure confirms that adequate molecular mass data and good recovery rates (≥97%) could be obtained with all grades.

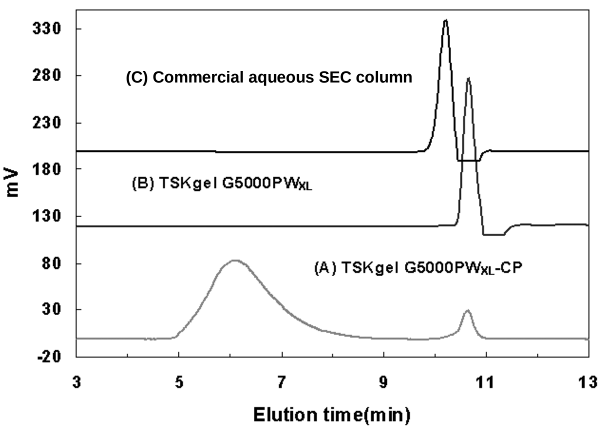

Fig. 9 Chromatograms of PAA-HCl using TSKgel G5000PW$_{XL}$-CP and conventional columns

Columns: (A) TSKgel G5000PW$_{XL}$-CP (7.8 mm I.D. x 30 cm)
(B) TSKgel G5000PW$_{XL}$ (7.8 mm I.D. x 30 cm)
(C) Commercial aqueous SEC column (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Poly(allylamine hydrochloride) (PAA-HCl) (3 g/L)
Injection volume: 100 μL

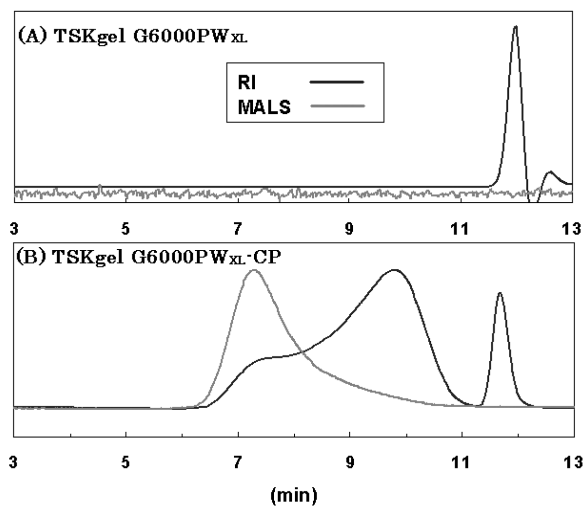

Fig. 10 Chromatograms of PAA-HCl using TSKgel G6000PW$_{XL}$-CP and TSKgel G6000PW$_{XL}$

Columns: (A) TSKgel G6000PW$_{XL}$ (7.8 mm I.D. x 30 cm)
(B) TSKgel G6000PW$_{XL}$-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: MALS (DAWN HELEOS), RI
Temperature: 40 ºC
Samples: Poly(allylamine hydrochloride) (PAA-HCl) (3 g/L)
Injection volume: 100 μL

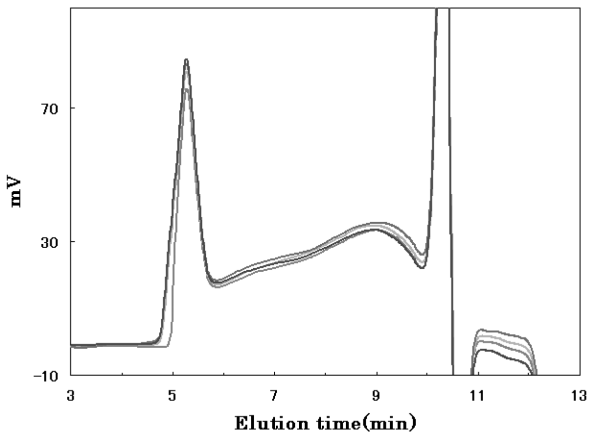

Fig. 11 Chromatograms of PAA-HCl in eluent with high salt concentration using a commercial aqueous SEC column and molecular mass data

Column: Commercial aqueous SEC column (7.8 mm I.D. x 30 cm)
Eluent: 0.5 mol/L acetic acid + 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Samples: Poly(allylamine hydrochloride) (PAA-HCl) (3 g/L)
Injection volume: 100 μL

Inject. No.	Mw	Area	Recovery (%)
1	151,000	8,866	91
2	156,000	9,545	98
3	3,300,000	9,650	99
4	3,480,000	9,742	99.6
5	3,510,000	9,778	

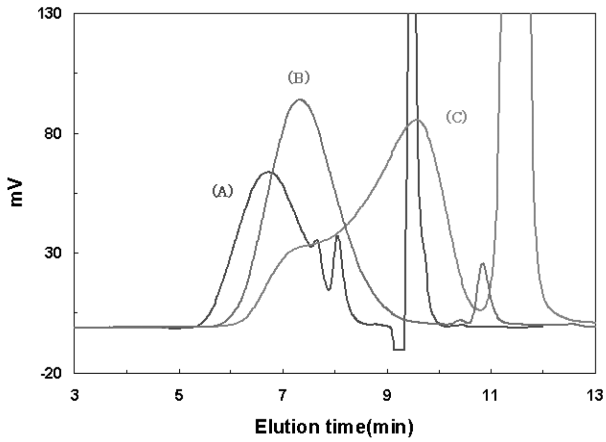

Fig. 12 Chromatograms of PAA-HCl by TSKgel PW_{XL}-CP series

Columns: (A) TSKgel G3000PW_{XL}-CP (7.8 mm I.D. x 30 cm)
(B) TSKgel G5000PW_{XL}-CP (7.8 mm I.D. x 30 cm)
(C) TSKgel G6000PW_{XL}-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Sample: Poly(allylamine hydrochloride) (PAA-HCl) (3 g/L) 100 μL
(A) PAA-HCl-01
(B) PAA-H-HCl
(C) PAA-HCl-10S

Grade	Mw	Recovery (%)
TSKgel G3000PW_{XL}-CP	6,500	100.2
TSKgel G5000PW_{XL}-CP	168,000	98.8
TSKgel G6000PW_{XL}-CP	455,000	97.4

5-2. Repeatability

Fig. 13 shows the results of an investigation of the repeatability (within a day) of analyses of PAA-HCl in a 0.1 mol/L aqueous solution of sodium nitrate, using the TSKgel G5000PWXL-CP.

From the first run, the sample showed high recovery rates, and the repeatability of chromatograms and average molecular mass (Mw) data analyzed by SEC were confirmed to be excellent.

On the other hand, as shown in **Fig. 14**, repeatability data in the TSKgel G5000PWXL indicated that even with repeated injection of samples under the same conditions, analyte peaks did not appear, clearly indicating that very strong adsorption was occurring.

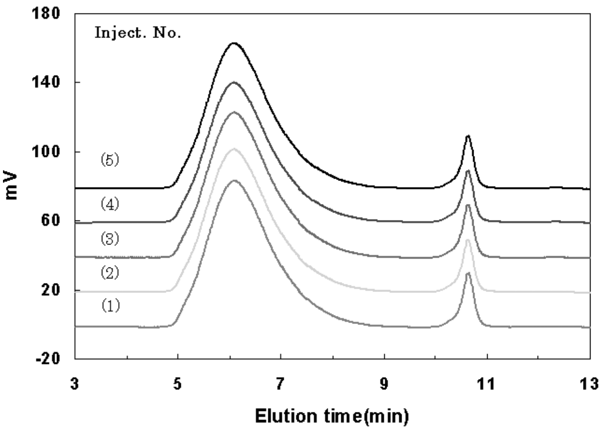

Fig. 13 Repeatability (within a day) of analyses of PAA-HCl using TSKgel G5000PWXL-CP

Column: TSKgel G5000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 °C
Samples: Poly(allylamine hydrochloride) (PAA-HCl) (0.3%)
Injection volume: 100 μL

Inject. No.	Mw	Recovery (%)
1	168,000	98.8
2	169,000	99.1
3	168,000	99.1
4	170,000	99.3
5	170,000	99.2

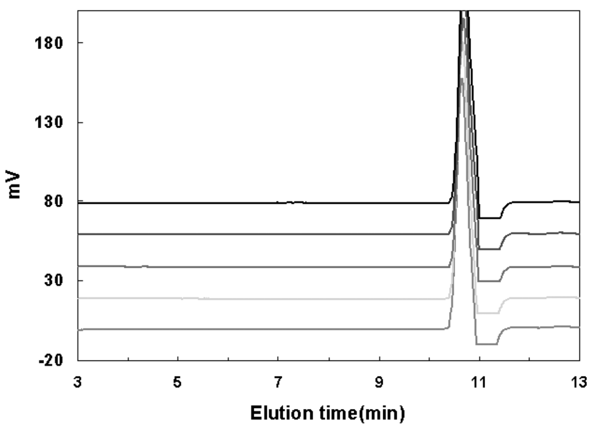

Fig. 14 Repeatability (within a day) of analyses of PAA-HCl using TSKgel G5000PWXL

Columns: TSKgel G5000PWXL (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 °C
Sample: Poly(allylamine hydrochloride) (PAA-HCl) (0.3%)
Injection volume: 100 μL

5-3. Application of various cationic polymers

Fig. 15 shows chromatograms of various polyethyleneimines and poly(allylamine hydrochloride) analyzed with the TSKgel G3000PWXL-CP. **Fig. 16** shows chromatograms of various poly(allylamine hydrochloride) with different molecular masss analyzed with the TSKgel G6000PWXL-CP.

Under each of these conditions, good chromatograms were obtained from the initial run, and samples were eluted in order according to molecular mass (largest molecules first), confirming that SEC elution was occurring normally.

Fig. 17 shows overlapping chromatograms of various cationic polymers with different properties and molecular mass measured in a system in which the TSKgel G6000PWXL-CP, TSKgel G5000PWXL-CP and TSKgel G3000PWXL-CP columns were connected in series.

The TSKgel PWXL-CP series shows an ability to perform good SEC analysis of various cationic polymers with different properties.

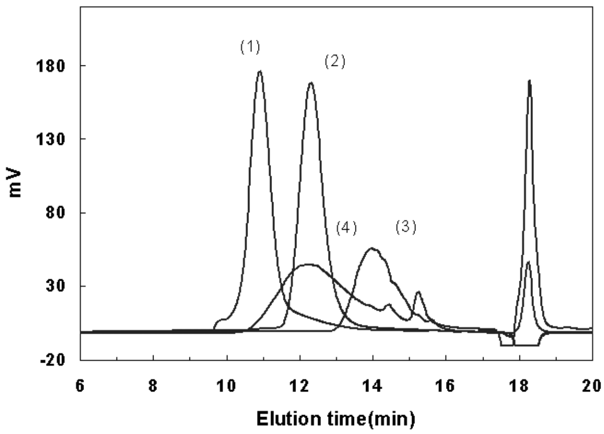

Fig. 15 Chromatograms of various cationic polymers using the TSKgel G3000PWXL-CP

Column: TSKgel G3000PWXL-CP (7.8 mm I.D. x 30 cm x 2)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Sample: Cationic polymers (3g/L) 100 µL
(1) Polyethyleneimine (10000)
(2) Polyethyleneimine (1800)
(3) Polyethyleneimine (300)
(4) Poly(allylamine hydrochloride)

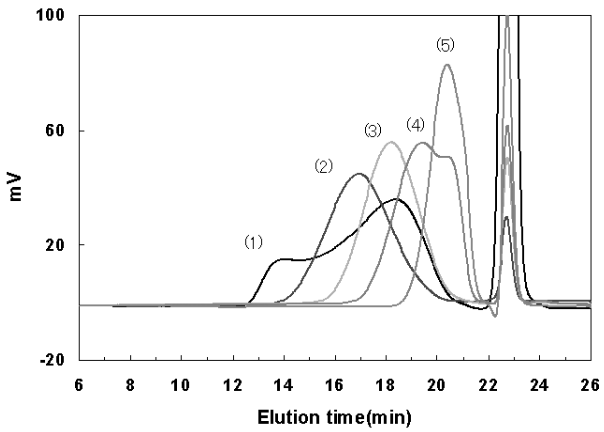

Fig. 16 Chromatograms of various poly (allylamine hydrochloride) using the TSKgel G6000PWXL-CP

Columns: TSKgel G6000PWXL-CP (7.8 mm I.D. x 30 cm x 2)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Sample: Poly(allylamine hydrochloride) (3g/L) 100 µL
(1) PAA-HCl
(2) PAA-H-HCl
(3) PAA-HCl-3L
(4) PAA-HCl-05
(5) PAA-HCl-01

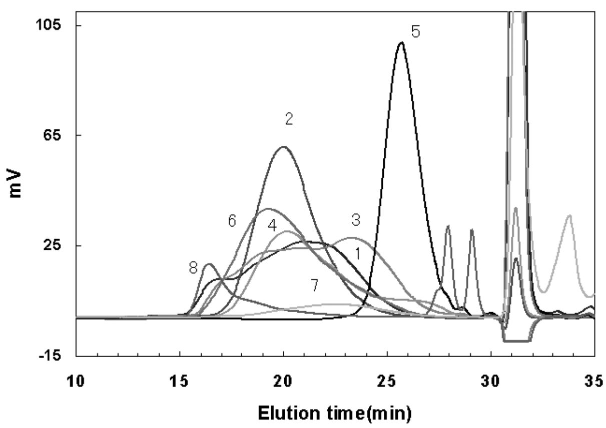

Fig. 17 Chromatograms of various cationic polymers using the TSKgel PWXL-CP series

Columns: TSKgel G(6000 + 5000 + 3000)PWXL-CP (7.8 mm I.D. x 30 cm x 3)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 25 ºC
Sample: Cationic polymers (3g/L) 100 µL
(1) Poly(allylamine hydrochloride) (PAA-HCl)
(2) Poly(allylamine hydrochloride) (PAA-H-10C)
(3) Polyethyleneimine
(4) Polydiallyl dimethyl ammonium chloride
(5) Polydiallyl dimethyl ammonium chloride/sulfur dioxide copolymer
(6) Polydiallyl dimethyl ammonium chloride/polyacrylamide copolymer
(7) Cationized dextran
(8) Chitosan

6. Analysis of absolute molecular mass of cationic polymers by SEC-MALS

The radius of inertia and absolute molecular mass of various cationic polymers were investigated using SEC-MALS (multi-angle light scattering detector) using TSKgel PWXL-CP columns.

6-1. Absolute molecular mass of polydiallyl dimethyl ammonium chloride

Fig. 18 shows absolute molecular mass data (MALS) and a chromatogram (RI) of polydiallyl dimethyl ammonium chloride (PDADM-NH_4Cl) analyzed by SEC-MALS (multi-angle light scattering detector) using the TSKgel G6000PWXL-CP and 0.1 mol/L aqueous solution of sodium nitrate as the eluent.

Fig. 19 shows the relationship between the absolute molecular mass and radius of inertia. It is clear from these results that the polymer components are being eluted in order starting with the largest molecules first, and that good SEC separation is occurring. The relationship between the absolute molecular mass and the radius of inertia is also good.

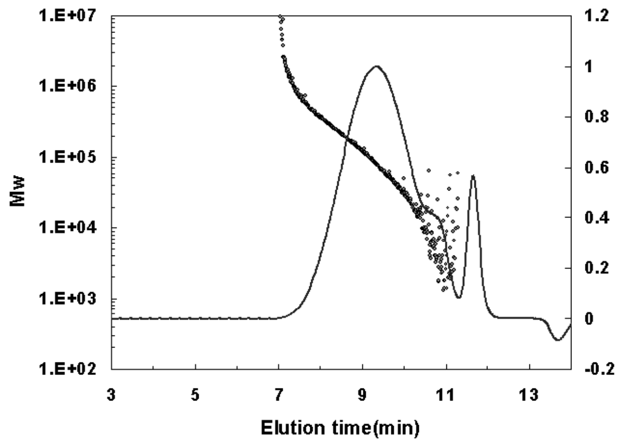

Fig. 18 Absolute molecular mass and chromatogram of elution of PDADM-NH_4Cl by the TSKgel G6000PWXL-CP

Column: TSKgel G6000PWXL-CP (7.8 mm I.D. x 30 cm)
Eluent: 0.1 mol/L sodium nitrate
Flow rate: 1.0 mL/min
Detection: MALS (DAWN HELEOS), RI
Temperature: 40 °C
Sample: Polydiallyl dimethyl ammonium chloride (1 g/L) 100 μL

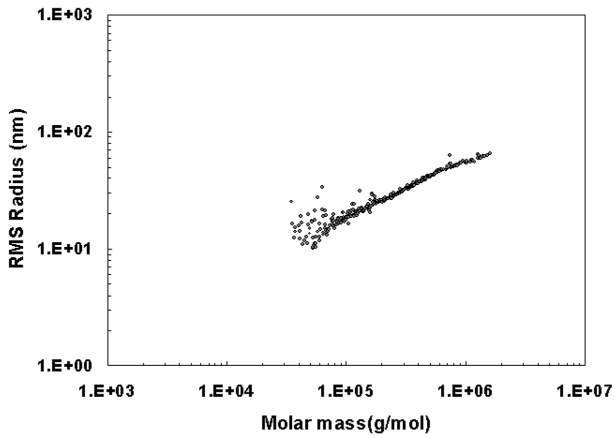

Fig. 19 Relationship between absolute molecular mass and radius of inertia of PDADM-NH_4Cl using the TSKgel G6000PWXL-CP

Same as Fig. 18
Sample: Polydiallyl dimethyl ammonium chloride (1 g/L) 100 μL

6-2. Absolute molecular mass of PDADM-NH_4Cl copolymer

The absolute molecular mass of copolymers of PDADM-NH_4Cl and acrylamide, and PDADM-NH_4Cl and sulfur dioxide copolymer was analyzed under the same conditions as **Fig. 19**.

Fig. 20 shows the relationship between absolute molecular mass and radius of inertia. It is clear that SEC separation is occurring normally.

As is clear from the data provided here, although the absolute molecular mass of each of these copolymers is approximately the same (about 200,000), the radius of inertia of the copolymer of PDADM-NH_4Cl and acrylamide is about 1.5 times as large.

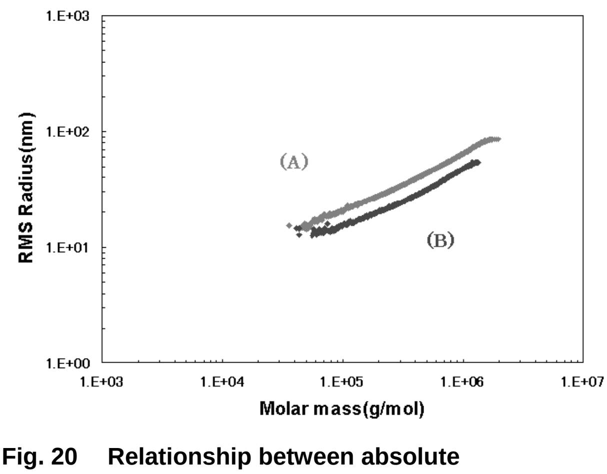

Fig. 20 Relationship between absolute molecular mass and radius of inertia of PDADM-NH_4CL copolymer using the TSKgel G6000PWXL-CP

Same as Fig. 21 **[Please verify this figure number.]**

Samples: (A) PDADM-NH_4Cl/polyacrylamide copolymer (1 g/L) 100 μL

(B) PDADM-NH_4Cl/sulfur dioxide copolymer (1 g/L) 100 μL

7. Conclusion

Until now it has been difficult to perform normal SEC analysis of aqueous cationic polymers, because it has been impossible to inhibit electrostatic interaction between the sample and the packing material.

To overcome such problems, we have improved the ionic characteristics on the surface of the packing material using a novel technique, and have confirmed that cationic polymers can now be analyzed with good repeatability, even in neutral eluents with low salt concentrations.

Data from analyses of absolute molecular mass by SEC-MALS also confirms that normal SEC separation is occurring with these columns.

The use of the TSKgel PWXL-CP series is expected to make it easier to analyze various cationic polymers.