



SEPARATION REPORT NO. 106

AQUEOUS SEC COLUMNS FOR ANALYSIS OF CATIONIC POLYMERS: TSKgel PWXL-CP SERIES

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1. Introduction

Polymer and silica matrices are widely used as packing materials in aqueous size exclusion chromatography (SEC) for analyzing molar 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 molar 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 containing 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 molar mass data is not always obtained.

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

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

2. Features

When the TSKgel PW_{XL} series columns are 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 molar mass distribution data to be obtained.

By minimizing the number of residual carboxyl groups on the surface of the packing material while not altering the pore structure of the corresponding TSKgel PW_{XL} columns, the TSKgel PW_{XL}-CP series columns provide excellent precision and high recovery rates for cationic polymers, even under conditions in which neutral eluents with low salt concentrations are used. An exception is TSKgel G3000PW_{XL}-CP in which the pore structure was altered to provide better separation performance for low molecular weight polymers as will be discussed in section 3-4.

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

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

	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	7μm	10μm	13μm
Molecular weight exclusion limit (PEO)	100,000	1,000,000	20,000,000*
Molecular weight 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 columns

Product name	Theoretical plates (N/ 30cm column)	Asymmetry factor	Column size (mm ID x cm)
TSKgel G3000PW _{XL} -CP	16,000	0.7 ~ 1.6	7.8 x 30
TSKgel G5000PW _{XL} -CP	10,000	0.7 ~ 1.6	7.8 x 30
TSKgel G6000PW _{XL} -CP	7,000	0.7 ~ 1.6	7.8 x 30

Analysis conditions

Eluent: 0.1mol/L sodium nitrate

Flow rate: 1.0mL/min (7.8mm ID x 30 cm)

Temperature: 25°C

Detection: RI

Injection volume: 20μL

Sample: ethylene glycol, 5g/L

3. Basic characteristics

3-1. Pore size characteristics

The TSKgel PW_{XL}-CP series columns, as shown in Tables 1 and 2, are provided in three grades with different molecular weight separation ranges capable of accommodating low to high molecular weight samples.

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

TSKgel G3000PW_{XL}-CP, the low molecular weight grade column, is suitable for analysis over a molecular weight separation range of 50,000 to 200. A molecular weight separation range of 500,000 to 400 was established for TSKgel G5000PW_{XL}-CP, and 10,000,000 to 1,000 for TSKgel G6000PW_{XL}-CP, the high molecular weight grade column. As a result, by connecting these columns in series depending on the molecular weight and molar mass distribution of the sample, TSKgel PW_{XL}-CP columns can accommodate a wide range of samples from high molecular weight polymers to low molecular weight oligomers.

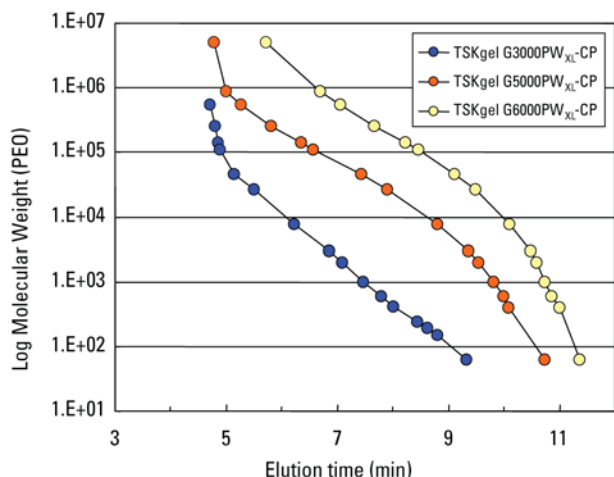


Figure 1 TSKgel PW_{XL}-CP series calibration curves

Columns: TSKgel PW_{XL}-CP series,
7.8mm ID x 30cm
Eluent: 0.1mol/L sodium nitrate
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 100μL
Samples: standard polyethylene oxide, polyethylene glycol and ethylene glycol

3-2. Sample injection volume and HETP

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

It is clear from this graph that when the injection volume exceeds 50μL, HETP begins to increase. Thus, the maximum sample injection volume is less than 50μL per TSKgel PW_{XL}-CP column.

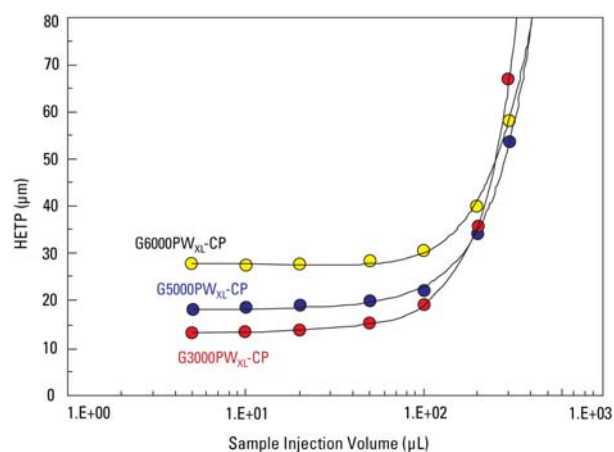


Figure 2 Relationship between HETP and sample injection volume in TSKgel PW_{XL}-CP series

Columns: TSKgel PW_{XL}-CP series,
7.8mm ID x 30cm x 2
Eluent: H₂O
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 5 to 500μL
Sample: ethylene glycol, 5g/L

3-3. Dependence of HETP on flow rate

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

As is well known, the optimal flow rate (minimum HETP) varies in accordance with the particle size of the packing material in the column. The optimum flow rate (minimum HETP) with TSKgel G3000PW_{XL}-CP, which has a 7 micron 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 TSKgel G5000PW_{XL}-CP, and to 0.4 to 0.7 mL/min with TSKgel G6000PW_{XL}-CP.

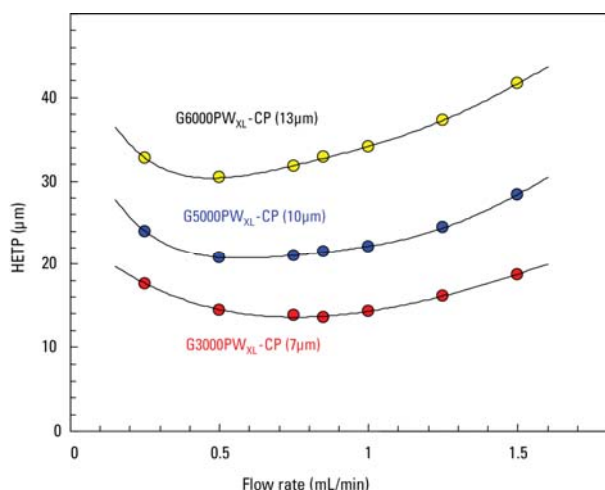


Figure 3 Relationship between HETP and flow rate in the TSKgel PW_{XL}-CP series

Columns: TSKgel PW_{XL}-CP series,
7.8mm ID x 30cm
Eluent: H₂O
Flow rate: 0.25 to 1.5mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 20μL
Sample: ethylene glycol, 5g/L

3-4. Separation performance

Although it was previously stated (see page 1) 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 TSKgel G3000PW_{XL}-CP, have been improved in comparison to the TSKgel G3000PW_{XL} column.

Figure 4 shows chromatograms of polyethylene glycol 200, a non-ionic oligomer, produced using the TSKgel G3000PW_{XL}-CP and the TSKgel G3000PW_{XL} columns.

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

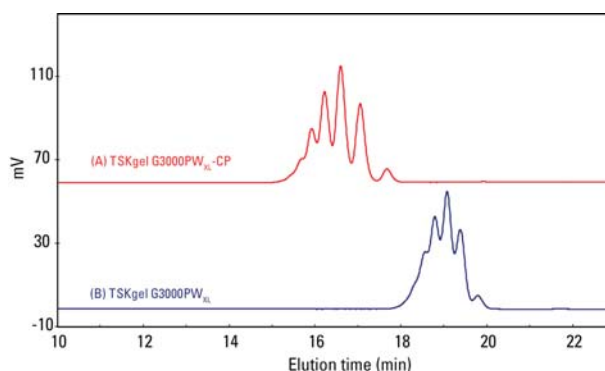


Figure 4 Separation of polyethylene glycol 200 using the TSKgel G3000PW_{XL}-CP and TSKgel G3000PW_{XL}

Columns: A: TSKgel G3000PW_{XL}-CP,
7.8mm ID x 30cm x 2
B: TSKgel G3000PW_{XL},
7.8mm ID x 30cm x 2
Eluent: H₂O
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 50μL
Sample: polyethylene glycol 200, 3g/L

4. Dependence of calibration curves on eluent

4-1. Temperature dependence

Figure 5 shows the results of an investigation of the temperature dependence of calibration curves with standard polyethylene oxide, using a TSKgel G5000PW_{XL}-CP column with 0.1mol/L sodium nitrate as the eluent. The figure confirms that there is no temperature dependence in the range from 25°C to 60°C.

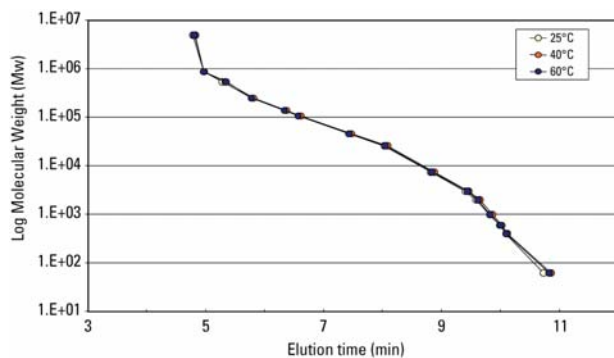


Figure 5 Dependence of calibration curves on temperature in TSKgel G5000PW_{XL}-CP

Column: TSKgel G5000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Temperature: 25°C, 40°C, 60°C
 Injection vol.: 100µL
 Samples: standard polyethylene oxide, polyethylene glycol, ethylene glycol

4-2. Dependence on pH

Figure 6 shows calibration curves produced with standard polyethylene oxide when the pH of the eluent (0.1mol/L acetate buffer) is varied using a TSKgel G5000PW_{XL}-CP column.

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

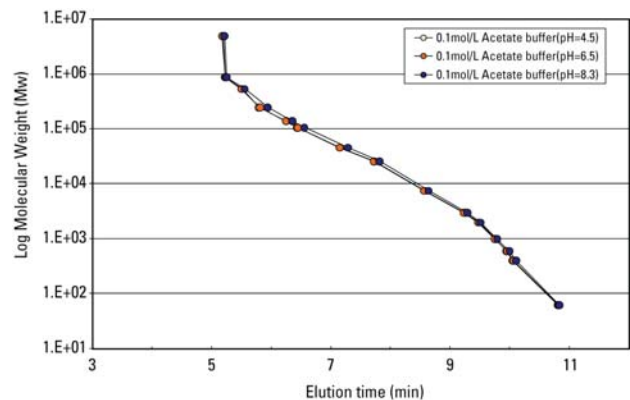


Figure 6 Dependence of calibration curves on pH of eluent in TSKgel G5000PW_{XL}-CP

Column: TSKgel G5000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L acetate buffer (pH = 4.5, 6.5, 8.3)
 Flow rate: 1.0mL/min
 Temperature: 25°C
 Injection vol.: 100µL
 Samples: standard polyethylene oxide, polyethylene glycol, ethylene glycol

4-3. Concentration of organic solvent

Figure 7 shows the results of an investigation of the effect of acetonitrile concentration on the calibration curve using the TSKgel G5000PW_{XL}-CP as the column, when acetonitrile is added to the eluent (0.1mol/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 towards lower molecular weights.

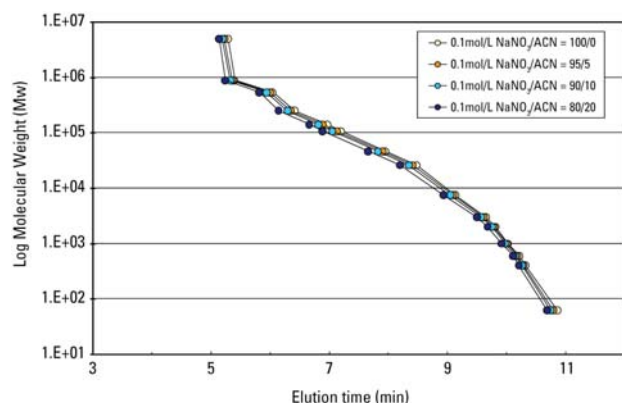


Figure 7 Effect of concentration of organic solvent on calibration curve of TSKgel G5000PW_{XL}-CP

Column: TSKgel G5000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate/acetonitrile (0/100 to 20/80)
 Flow rate: 1.0mL/min
 Temperature: 25°C
 Injection vol.: 100 µL
 Samples: standard polyethylene oxide, polyethylene glycol, ethylene glycol

4-4. Flow rate dependency

Figure 8 shows the results of an investigation of the dependency of standard polyethylene oxide calibration curves on the flow rate using the TSKgel G6000PW_{XL}-CP column and 0.1mol/L sodium nitrate as eluent. The results confirmed that there were no marked changes in the calibration curve over a range of flow rates from 0.25 to 1.0mL/min.

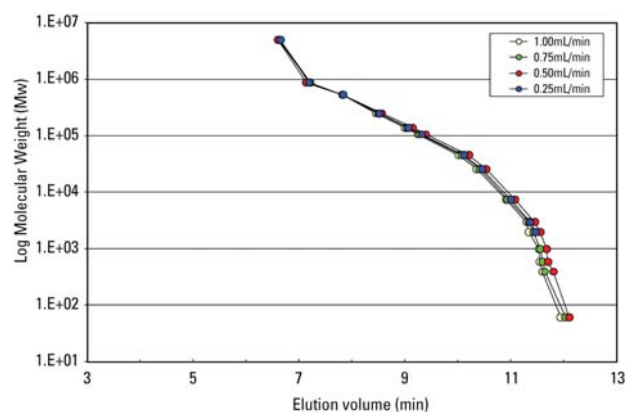


Figure 8 Dependence of TSKgel G6000PW_{XL}-CP calibration curves on flow rate

Column: TSKgel G6000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 0.25 to 1.0mL/min
 Temperature: 25°C
 Injection vol.: 100µL
 Samples: standard polyethylene oxide, polyethylene glycol, ethylene glycol

5. Elution characteristics of cationic polymers

5-1. Comparison of elution using commercial SEC column

Figure 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 (0.1mol/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 from the PW_{XL} and the commercial aqueous SEC columns. However, the chromatogram obtained with the TSKgel G5000PW_{XL}-CP column showed good elution of the sample.

Figure 10 shows chromatograms obtained analyzing PAA-HCl with a multi-angle light scattering detector (MALS) using TSKgel G6000PW_{XL}-CP and TSKgel G6000PW_{XL} columns, under the same solvent conditions as in Figure 9 (0.1mol/L sodium nitrate).

The figure verifies that even with MALS, PAA-HCl fails to elute from the TSKgel G6000PW_{XL} column due to adsorption and thus no peak is detected.

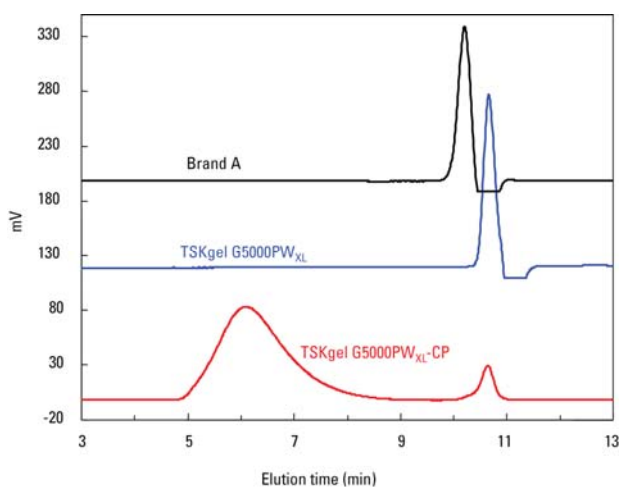


Figure 9 Chromatograms of PAA-HCl using TSKgel G5000PW_{XL}-CP and conventional columns

Columns: A: TSKgel G5000PW_{XL}-CP, 7.8mm ID x 30cm
 B: TSKgel G5000PW_{XL}, 7.8mm ID x 30cm
 C: Commercial aqueous SEC column, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: RI
 Temperature: 25°C
 Injection vol.: 100μL
 Sample: poly(allylamine hydrochloride) (PAA-HCl), 3g/L

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

Figure 11 shows a chromatogram produced by repeated analyses of PAA-HCl using a commercial aqueous SEC column in 0.5mol/L acetate + 0.1mol/L sodium nitrate. The figure confirms that although the cationic polymer could be eluted under these solvent conditions, the recovery rates were as low as 91% for the first injection and increased with subsequent injections up to 99.6%. It indicates that strong electrostatic interaction occurs between cationic polymers and packing materials, especially at the initial numbers of injections.

Figure 12 shows the results of an investigation of the elution characteristics of PAA-HCl using each of the TSK-GEL PW_{XL}-CP series columns in 0.1mol/L sodium nitrate. Clearly, the separation can be achieved under these milder salt conditions. Sample recovery rates obtained with each TSKgel PW_{XL}-CP column type were good (≥97%) from the first injection.

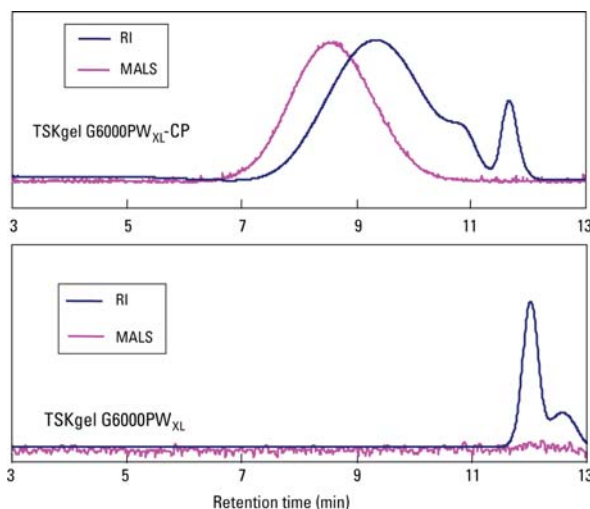


Figure 10 Chromatograms of PAA-HCl using TSKgel G6000PW_{XL}-CP and TSKgel G6000PW_{XL} columns

Columns: A: TSKgel G6000PW_{XL}, 7.8mm ID x 30cm
 B: TSKgel G6000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: MALS (DAWN HELEOS), RI
 Temperature: 40°C
 Injection vol.: 100μL
 Sample: poly(allylamine hydrochloride) (PAA-HCl), 3g/L

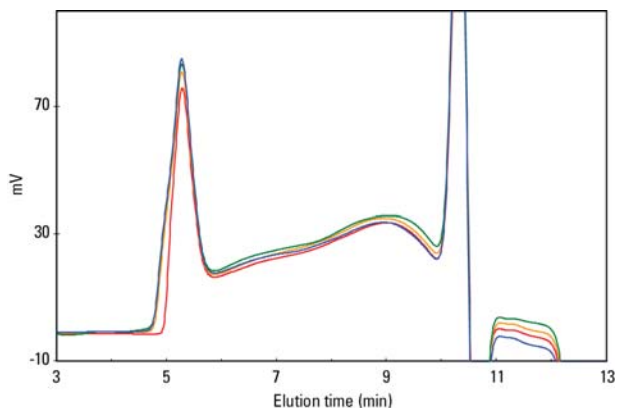


Figure 11 Chromatograms of PAA-HCl in eluent with high salt concentration using a commercial aqueous SEC column and molecular weight data

Column: Commercial aqueous SEC column, 7.8mm ID x 30cm
 Eluent: 0.5mol/L acetic acid + 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: RI
 Temperature: 25°C
 Injection vol.: 100µL
 Sample: poly(allylamine hydrochloride) (PAA-HCl), 3g/L

Inject. No.	Area	Recovery (%)
1	8,866	91
2	9,545	98
3	9,650	99
4	9,742	99.6
5	9,778	

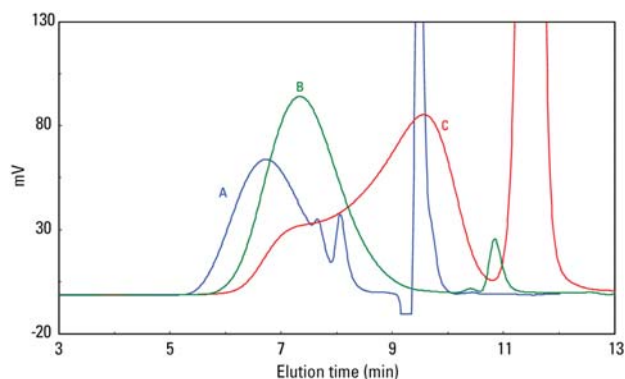


Figure 12 Chromatograms of PAA-HCl by TSKgel PW_{xl}-CP series

Columns: A: TSKgel G3000PW_{xl}-CP, 7.8mm ID x 30cm
 B: TSKgel G5000PW_{xl}-CP, 7.8mm ID x 30cm
 C: TSKgel G6000PW_{xl}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: RI
 Temperature: 25°C
 Injection vol.: 100µL
 Samples: poly(allylamine hydrochloride) (PAA-HCl), 3g/L

A. PAA-HCl-01
 B. PAA-H-HCl
 C. PAA-HCl-10S

Column	Recovery (%)
TSKgel G3000PW _{xl} -CP	100.2
TSKgel G5000PW _{xl} -CP	98.8
TSKgel G6000PW _{xl} -CP	97.4

5-2. Repeatability

Figure 13 shows the results of an investigation of the precision (within day) of analyses of PAA-HCl in 0.1mol/L sodium nitrate, using a TSKgel G5000PW_{XL}-CP column.

From the first run, the sample showed high recovery and the precision and average molecular weight (Mw) data analyzed by SEC were confirmed to be excellent.

On the other hand, when performing the same experiment on a G5000PW_{XL} column the polymer failed to elute, *even after repeated injections*, from the column due to strong adsorption onto the matrix.

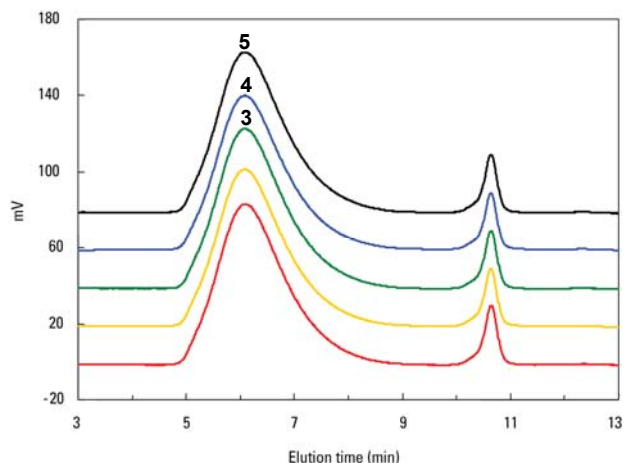


Figure 13 Repeatability (within a day) of analyses of PAA-HCl using TSKgel G5000PW_{XL}-CP

Column: TSKgel G5000PW_{XL}-CP, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: RI
 Temperature: 25°C
 Injection vol.: 100µL
 Sample: poly(allylamine hydrochloride) (PAA-HCl), 0.3%

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

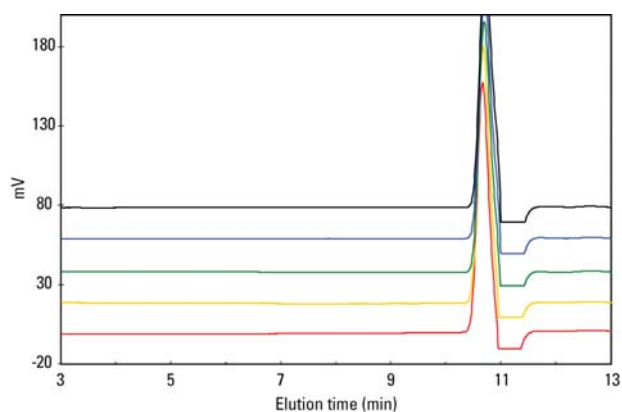


Figure 14 Repeatability (within a day) of analyses of PAA-HCl using TSKgel G5000PW_{XL}

Column: TSKgel G5000PW_{XL}, 7.8mm ID x 30cm
 Eluent: 0.1mol/L sodium nitrate
 Flow rate: 1.0mL/min
 Detection: RI
 Temperature: 25°C
 Injection vol.: 100µL
 Sample: poly(allylamine hydrochloride) (PAA-HCl), 0.3%

5-3. Application of various cationic polymers

Figure 15 shows chromatograms of various poly(ethylene imines) and poly(allylamine hydrochloride) analyzed with a TSKgel G3000PW_{XL}-CP column. Figure 16 shows chromatograms of various poly(allylamine hydrochloride) with different molecular weight analyzed with a TSKgel G6000PW_{XL}-CP column.

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

Figure 17 shows overlapping chromatograms of various cationic polymers with different properties and molecular weight measured in a system in which TSKgel G6000PW_{XL}-CP, TSKgel G5000PW_{XL}-CP and TSKgel G3000PW_{XL}-CP columns were connected in series.

The chromatograms in Figure 17 demonstrate good SEC behavior on TSKgel PW_{XL}-CP series columns for a variety of cationic polymers with different chemical properties.

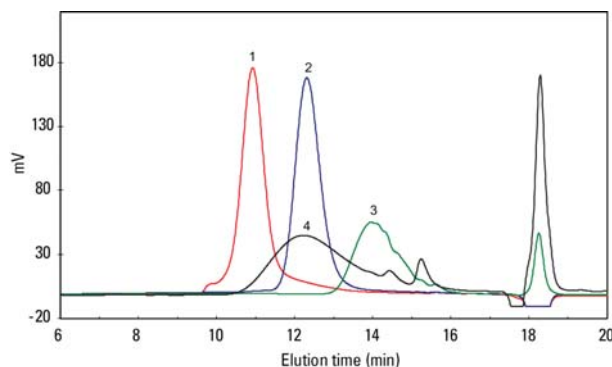


Figure 15 Chromatograms of various cationic polymers using TSKgel G3000PW_{XL}-CP

Column: TSKgel G3000PW_{XL}-CP,
7.8mm ID x 30cm x 2
Eluent: 0.1mol/L sodium nitrate
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 100µL
Samples: cationic polymers, 3g/L
1. polyethyleneimine (10,000)
2. polyethyleneimine (1,800)
3. polyethyleneimine (300)
4. poly(allylamine hydrochloride)

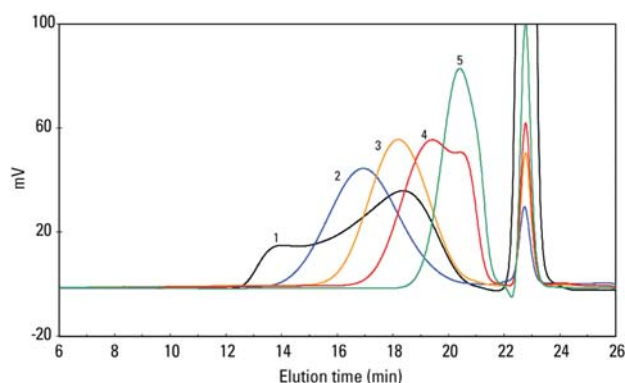


Figure 16 Chromatograms of various poly(allylamine hydrochloride) using TSKgel G6000PW_{XL}-CP

Column: TSKgel G6000PW_{XL}-CP,
7.8mm ID x 30cm x 2
Eluent: 0.1mol/L sodium nitrate
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 100µL
Samples: poly(allylamine hydrochloride), 3g/L

1. PAA-HCl
2. PAA-H-HCl
3. PAA-HCl-3L
4. PAA-HCl-05
5. PAA-HCl-01

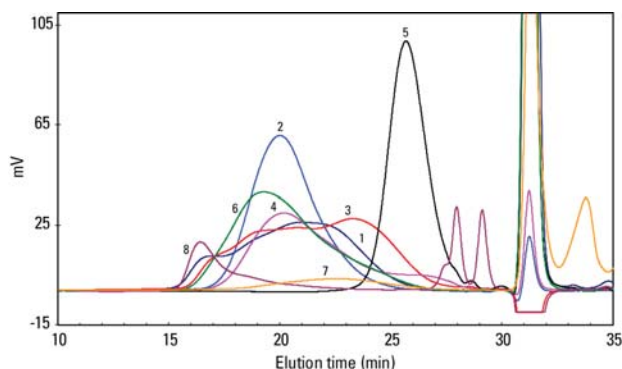


Figure 17 Chromatograms of various cationic polymers using the TSKgel PW_{XL}-CP series

Columns: TSKgel G(6000 + 5000 + 3000)PW_{XL}-CP,
7.8mm ID x 30cm x 3
Eluent: 0.1mol/L sodium nitrate
Flow rate: 1.0mL/min
Detection: RI
Temperature: 25°C
Injection vol.: 100µL
Samples: cationic polymers, 3g/L
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. cationic dextran
8. chitosan

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

The radius of gyration and absolute molecular weight of various cationic polymers were investigated using SEC-MALS (multi-angle light scattering detector) using TSKgel PW_{XL}-CP columns.

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

Figure 18 shows absolute molecular weight data (MALS) and a chromatogram (RI) of polydiallyl dimethyl ammonium chloride (PDADM-NH₄Cl) analyzed by SEC-MALS using TSKgel G6000PW_{XL}-CP and 0.1 mol/L sodium nitrate.

Figure 19 shows the relationship between the absolute molecular weight and radius of gyration. It is clear from these results that the polymer components are being eluted starting with the largest molecules first and that good SEC separation is taking place. The relationship between the absolute molecular weight and the radius of gyration is also good.

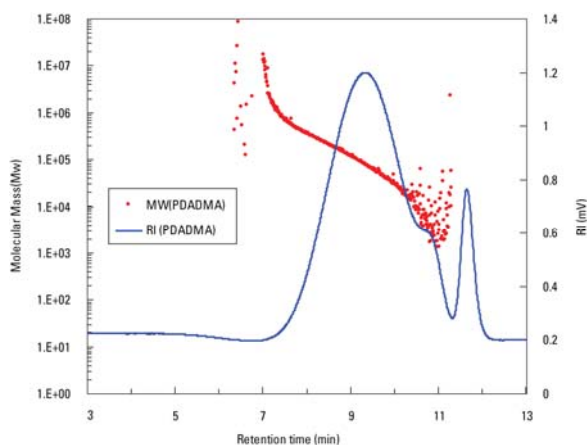


Figure 18 Absolute molecular weight and chromatogram of elution of PDADM-NH₄Cl using TSKgel G6000PW_{XL}-CP

Column: TSKgel G6000PW_{XL}-CP, 7.8mm ID x 30cm
Eluent: 0.1mol/L sodium nitrate
Flow rate: 1.0mL/min
Detection: MALS (DAWN HELEOS), RI
Temperature: 40°C
Injection vol.: 100μL
Sample: polydiallyl dimethyl ammonium chloride, 1g/L

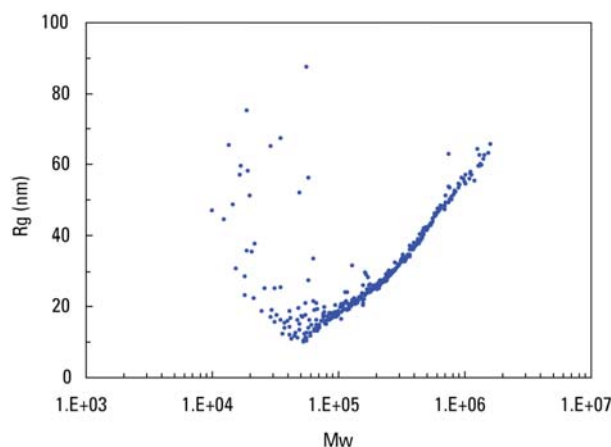


Figure 19 Relationship between absolute molecular weight and radius of gyration of PDADM-NH₄Cl using TSKgel G6000PW_{XL}-CP

Conditions as in Figure 18

6-2. Absolute molecular weight of PDADM-NH₄Cl copolymer

The absolute molecular weight of copolymers of PDADM-NH₄Cl and acrylamide, and PDADM-NH₄Cl and sulfur dioxide copolymer, were analyzed under the same conditions as in Figure 19.

Figure 20 shows the relationship between absolute molecular weight and radius of gyration. It is clear that regular SEC separation is taking place.

As is clear from the data provided here, although the absolute molecular weight of each of these copolymers is approximately the same (about 200,000), the radius of gyration of the copolymer of PDADM-NH₄Cl and acrylamide is about 1.5 times larger than that of PDADM-NH₄Cl polymer.

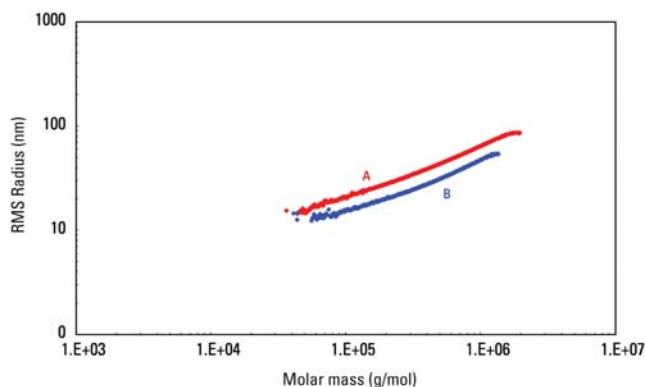


Figure 20 Relationship between absolute molecular weight and radius of gyration of PDADM-NH₄CL copolymer using TSKgel G6000PW_{XL}-CP

Conditions as in Figure 19

Injection vol.: 100 μ L

Samples: A. PDADM-NH₄Cl/polyacrylamide copolymer, 1g/L
B. PDADM-NH₄Cl/sulfur dioxide copolymer, 1g/L

7. Conclusions

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

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

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

The use of the TSKgel PW_{XL}-CP series columns is expected to make it easier to analyze various cationic polymers.