Basic Properties of Novel Aqueous SEC Columns Having Linear Calibration Curves

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Introduction

- Size exclusion chromatography (SEC) is a tool used for the characterization of polymers such as water-soluble polymers and plastics in various fields. SEC in aqueous or organic mobile phase is used for the determination of molecular information of polymer compounds based on a calibration curve.
- Polymer samples have a wide range of molecular mass, thus columns packed with particles having different pore sizes are required to analyze these types of samples. When two (or more) columns of different pore sizes are coupled together, one or more mismatches may show up as inflection points in the chromatogram when the slopes of the individual calibration curves are not identical. To prevent such a mismatch, columns ideally should be packed with particles containing a wide range of pore sizes. So-called “multipore” type columns are designed to improve the linearity of the calibration curve over a wide range of molecular weights.
- Recently TOSOH developed TSK-GEL® SuperMultiporePW columns: a series of novel multipore columns for aqueous SEC analysis packed with spherical monodisperse polymethacrylate particles. In addition, we also developed a high resolution semi-micro column, the TSKgel® SuperOligoPW column, for fast analysis of low molecular weight water-soluble polymers.
- In this poster, we report on the basic properties of these new TSK-GEL SEC columns and show elution profile comparisons of synthetic polymers on the newly developed columns and conventional columns.

Experimental

Columns – Tosoh Corporation (Japan)

Semi-micro Multipore:
- TSKgel SuperMultiporePW-N, PW-M, PW-H, 6.0mm ID x 15cm

Semi-micro:
- TSKgel SuperOligoPW, 6.0mm ID x 15cm

Conventional:
- TSKgel G-Oligo-PW, 7.8mm ID x 30cm
- TSKgel G3000PW$_{XL}$, G2500PW$_{XL}$, 6.0mm ID x 15cm

Column – Competitive
- Competitive column, 6.0mm ID x 15cm

Instrumentation

Instrument: EcoSEC® GPC system (Tosoh)
Data processing: EcoSEC-WS (Tosoh)

Chemicals and Reagents

Eluent and sample solutions were prepared with a Milli-Q® water purification system.
Standard samples of PEO and PEG were obtained from Tosoh. Other synthetic polymers and reagents were purchased from Wako Pure Chemical (Osaka) and other suppliers.

Preparation of Sample Solution

PEO and PEG standards were dissolved in water at concentrations of 0.5-1.0g/L. Synthetic polymers were dissolved in eluent at concentrations of 3.0-5.0g/L prior to use.

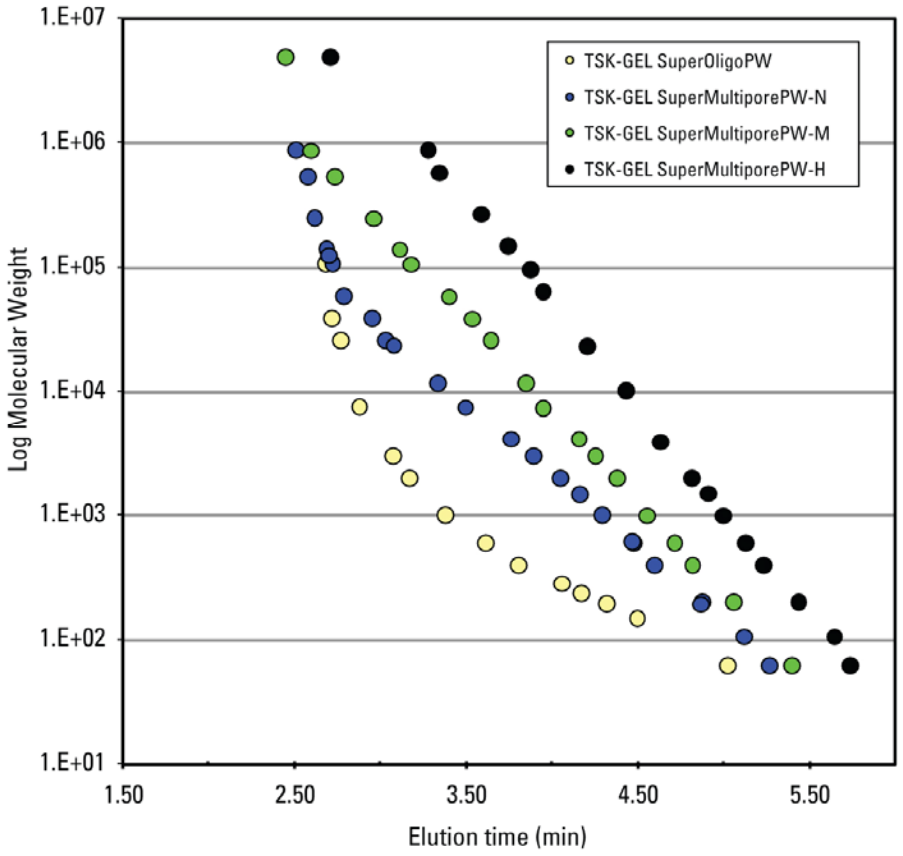
Properties of TSK-GEL SuperOligoPW and SuperMultiporePW columns

Table 1: Specifications

	TSKgel SuperOligoPW	TSKgel SuperMultiporePW-N	TSKgel SuperMultiporePW-M	TSKgel SuperMultiporePW-H
Base material	Polymethacrylate	Polymethacrylate	Polymethacrylate	Polymethacrylate
Particle size (µm)	3 (monodisperse)	4 (monodisperse)	5 (monodisperse)	8 (6 - 10)
Max. exclusion limit MW (PEO,PEG/H_2O)	4,000 - 8,000	100,000 - 150,000	600,000 - 1,500,000	
Range of applicable MW (PEO,PEG/H_2O)	100 - 3,000	300 - 50,000	500 - 1,000,000	1,000 - 10,000,000
Theoretical plates/column	>16,000	>16,000	>12,000	>7,000
Pressure drop (MPa)	4 - 6	4 - 6	2 - 4	1 - 2
Column size	6.0mm ID x 15cm	6.0mm ID x 15cm	6.0mm ID x 15cm	6.0mm ID x 15cm
Guard column	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm	4.6mm ID x 3.5cm

Properties of TSK-GEL SuperOligoPW and SuperMultiporePW columns

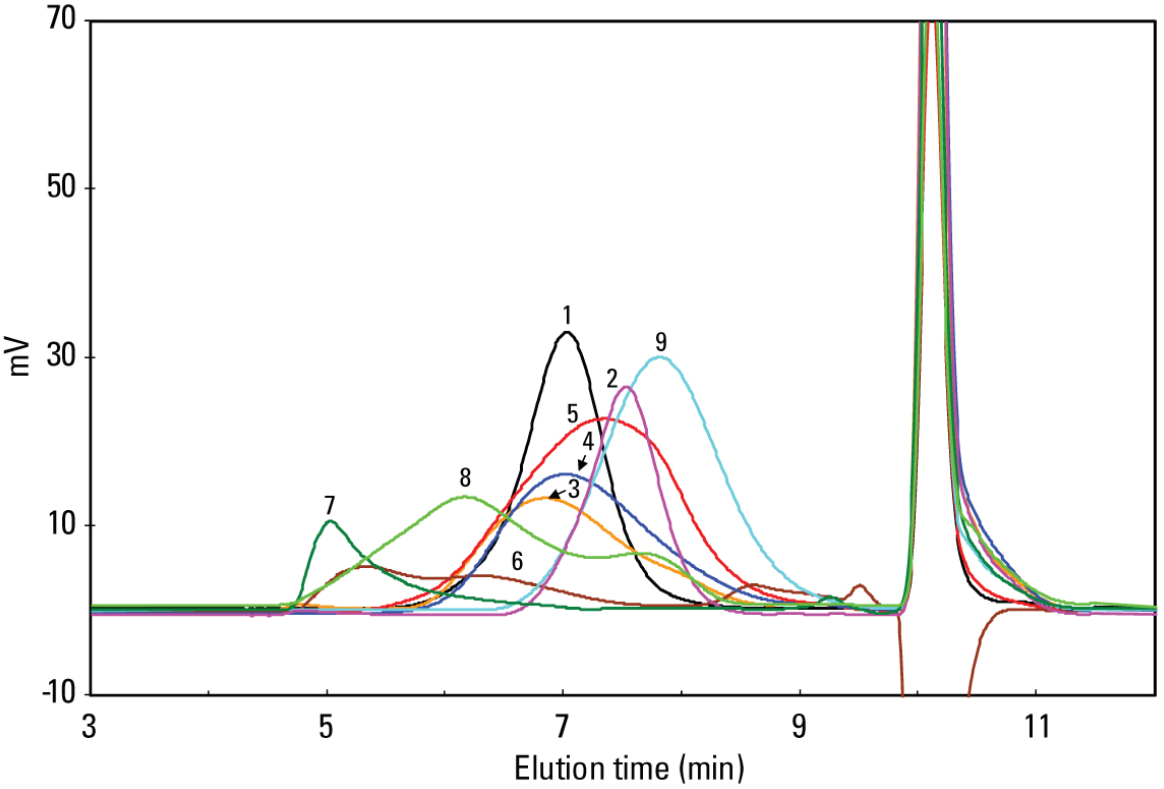
Figure 1: Calibration curves of aqueous SEC columns



Columns:	TSKgel SuperOligoPW, 6.0mm ID x 15cm TSKgel SuperMultiporePW-N, 6.0mm ID x 15cm TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm TSKgel SuperMultiporePW-H, 6.0mm ID x 15cm
Mobile phase:	H_2O
Flow rate:	0.60mL/min
Detection:	RI
Temperature:	25°C
Samples:	PEO, PEG and ethylene glycol

Elution profiles of water-soluble polymers

Figure 2: Separation of water-soluble polymers

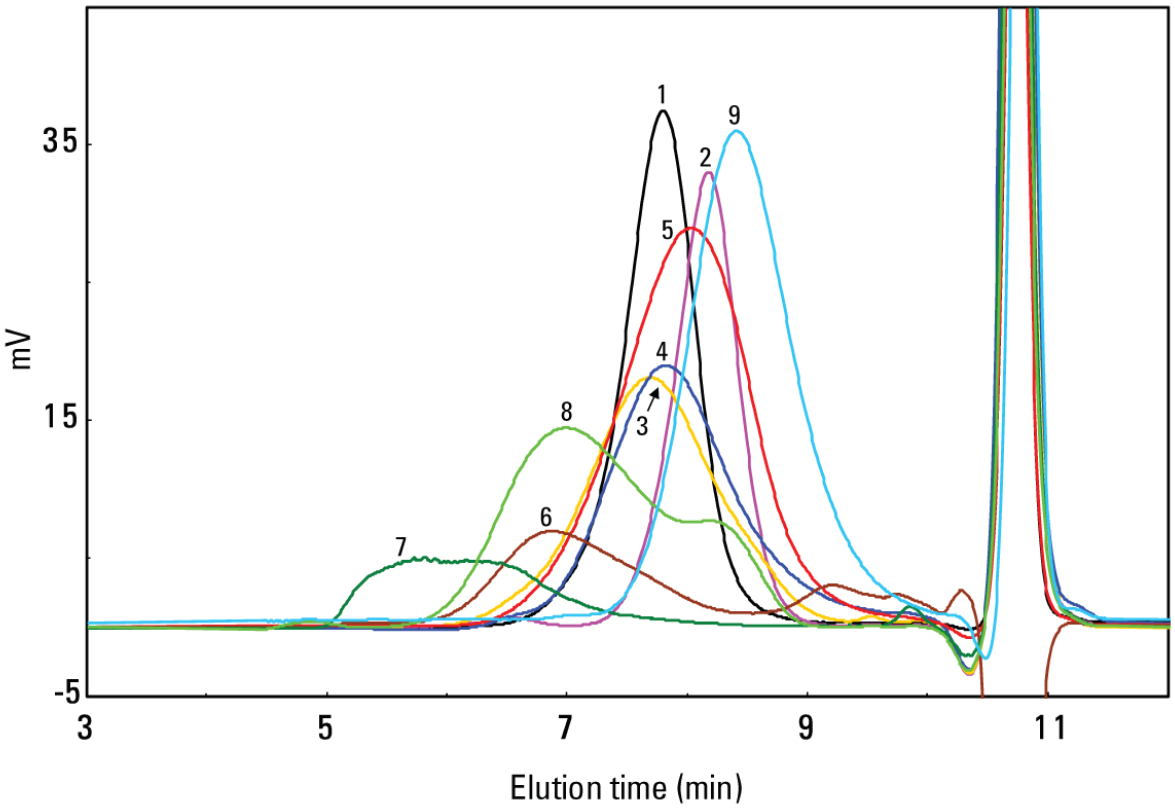


Column:	TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm x 2
Mobile phase:	100mmol/L $NaNO_3$
Flow rate:	0.6mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	20µL
Samples:	1. arabic gum
	2. chondroitin sulfate sodium salt
	3. carboxymethyl cellulose
	4. hydroxypropyl cellulose
	5. dextran(EH-7114)
	6. dextran(200K)
	7. polyacrylic acid sodium salt
	8. sulfopropyl methacrylate/poly(acrylic acid)
	9. polyvinylpyrrolidone(K-30)

MW results are summarized in Table 2.

Elution profiles of water-soluble polymers

Figure 3: Separation of water-soluble synthetic polymers, cont.

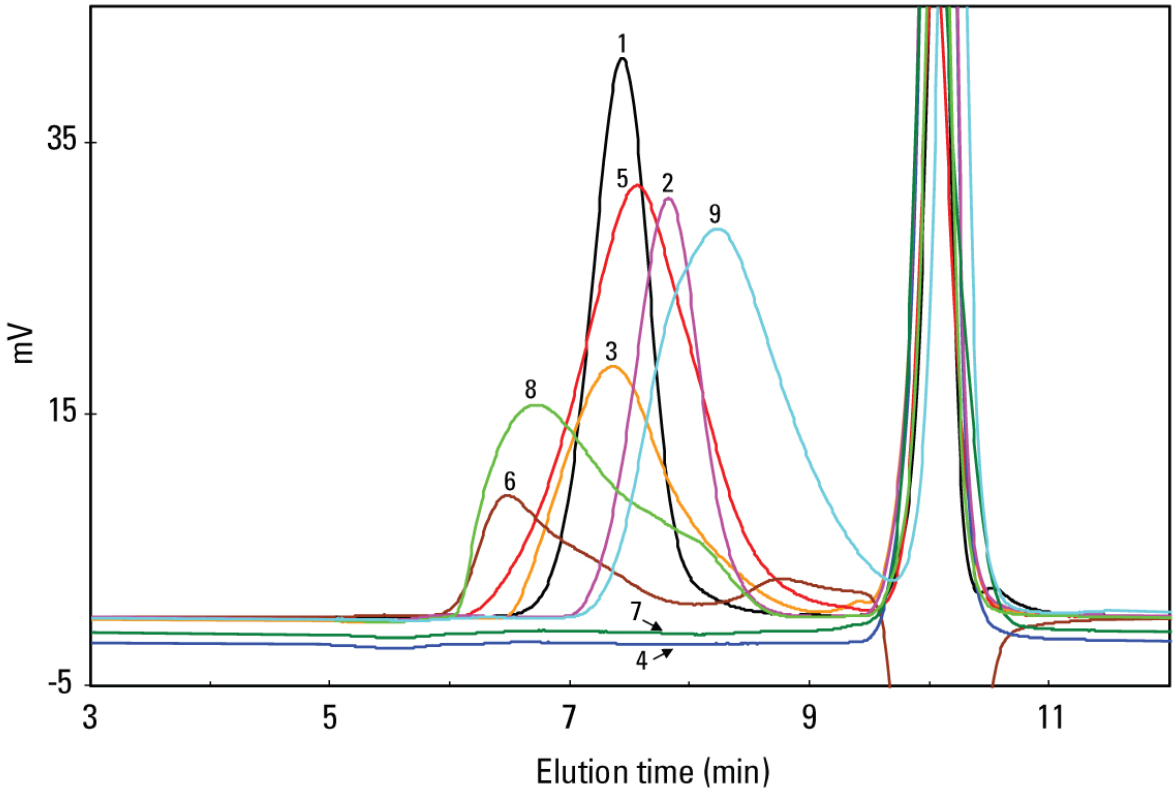


Column: TSKgel SuperMultiporePW-H, 6.0mm ID x 15cm x 2
Mobile phase: 100mmol/L $NaNO_3$
Flow rate: 0.6mL/min
Detection: RI
Temperature: 40°C
Injection vol.: 20µL
Samples:
1. arabic gum
2. chondroitin sulfate sodium salt
3. carboxymethyl cellulose
4. hydroxypropyl cellulose
5. dextran(EH-7114)
6.dextran(200K)
7. polyacrylic acid sodium salt
8. sulfopropyl methacrylate/poly(acrylic acid)
9. polyvinylpyrrolidone(K-30)

MW results are summarized in Table 2.

Elution profiles of water-soluble polymers

Figure 4: Separation of water-soluble synthetic polymers, cont.



Column: Competitive column, 6.0mm ID x 15cm x 2
Mobile phase: 100mmol/L $NaNO_3$
Flow rate: 0.6mL/min
Detection: RI
Temperature: 40°C
Injection vol.: 20µL
Samples:
1. arabic gum
2. chondroitin sulfate sodium salt
3. carboxymethyl cellulose
4. hydroxypropyl cellulose
5. dextran(EH-7114)
6. dextran(200K)
7. polyacrylic acid sodium salt
8. sulfopropyl methacrylate/poly(acrylic acid)
9. polyvinylpyrrolidone(K-30)

MW results are summarized in Table 2.

Analysis of water-soluble polymers

Table 2: Comparison of molecular weights of water soluble polymers

Sample polymer	TSKgel SuperMultiporePW-M	TSKgel SuperMultiporePW-H	Competitive column
(1) Arabic gum	126,000	103,000	106,000
(2) Chondroitin sulfate sodium salt	48,000	41,000	47,000
(3) Carboxymethyl cellulose	134,000	150,000	120,000
(4) Hydroxypropyl cellulose	108,000	91,000	*
(5) Dextran(EH-7114)	105,000	95,000	118,000
(6) Dextran(200K)	965,000	473,000	486,000
(7) Polyacrylic acid sodium salt	4,980,000	2,120,000	*
(8) Sulfopropyl methacrylate/Poly (acrylic acid)	2,130,000	395,000	340,000
(9) Polyvinylpyrrolidone(K-30)	31,000	29,000	22,000

*Not eluted

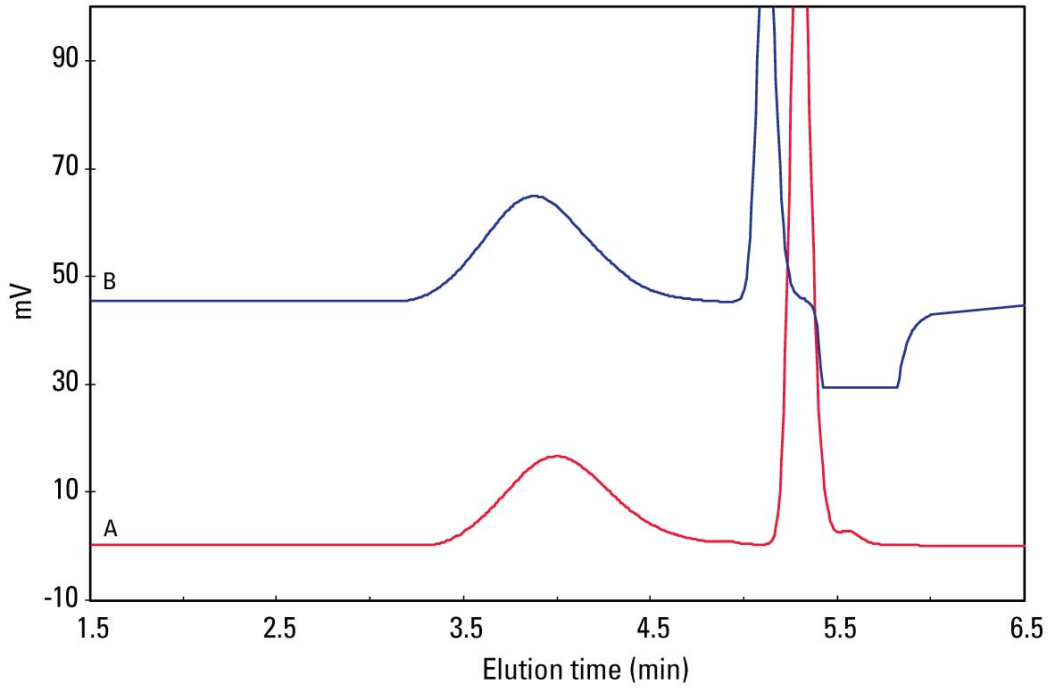
Hydroxypropyl cellulose and polyacrylic acid sodium salt were irreversibly adsorbed on the competitive column due to strong hydrophobic and ionic interactions.

Higher molecular masses were found on the TSK-GEL SuperMultiporePW columns compared to the competitive column.

For the larger MW samples (No. 6-8), we obtained higher molecular masses on the SuperMultiporePW-M column because of the smaller exclusion limit of this column.

Effect of separation conditions on the analysis of PVP

Figure 5: Effect of ACN content on molecular weight



Column:	TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm x 2
Mobile phase:	A:100mmol/L $NaNO_3$
	B:100mmol/L $NaNO_3$/ACN = 80/20(v/v)
Flow rate:	0.6mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	20µL
Sample:	PVP(K-30)

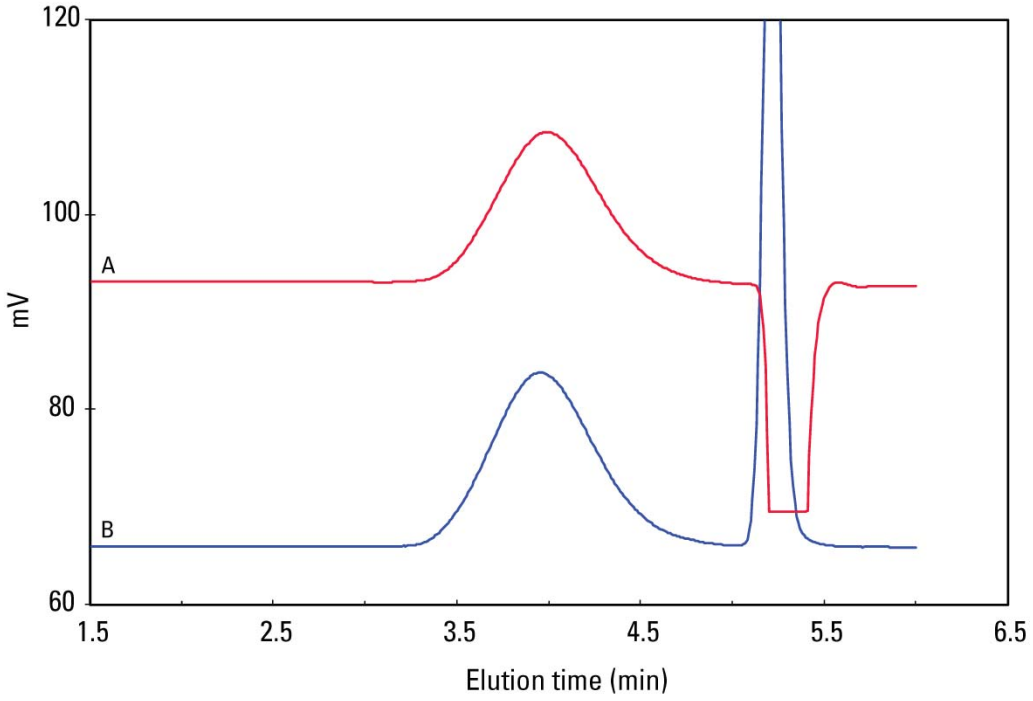
Mobile phase	100mmol/L $NaNO_3$/ACN	MW
A	100/0	19,000
B	80/20	22,000

Adding ACN to the mobile phase slightly increases the molecular mass of the PVP (polyvinylpyrrolidone) polymer as acetonitrile cuts down on hydrophobic interaction between the PVP and the base material.

To obtain accurate molecular weights of hydrophobic polymers, up to 50% organic solvent may be added to the mobile phase.

Effect of separation conditions on the analysis of PVP

Figure 6: Effect of salt concentration on molecular weight



Column:	TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm x 2
Mobile phase:	A: 200mmol/L $NaNO_3$
	B: 50mmol/L $NaNO_3$
Flow rate:	0.6mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	20µL
Sample:	PVP(K-30)

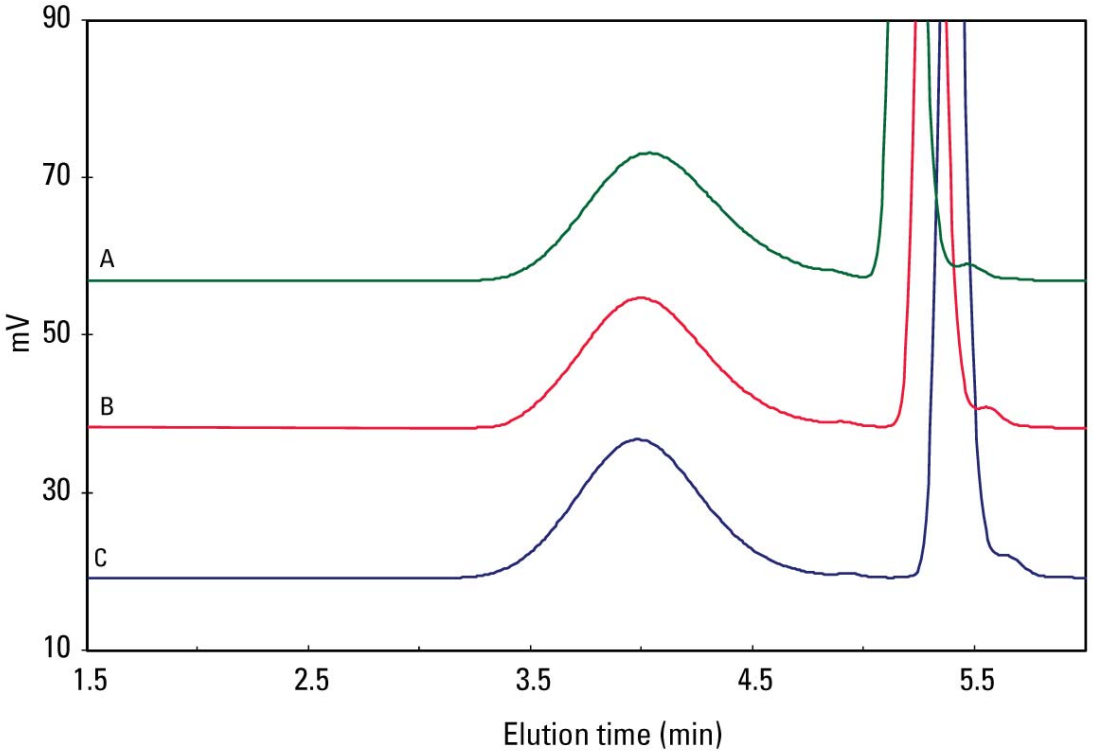
Mobile phase	Salt concentration	MW
A	200mmol/L	19,000
B	50mmol/L	21,000

Salting out of PVP polymer is observed at higher salt concentrations due to favored hydrophobic interaction.

50-100mmol/L of salt in the mobile phase is recommended to prevent ionic interaction without increasing hydrophobic interaction.

Effect of separation conditions on the analysis of PVP

Figure 7: Effect of temperature on molecular weight



Column: TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm x 2
Mobile phase: 100mmol/L $NaNO_3$
Flow rate: 0.6mL/min
Detection: RI
Temperature: 25°C, 40°C, 60°C
Injection vol.: 20µL
Sample: PVP(K-30)

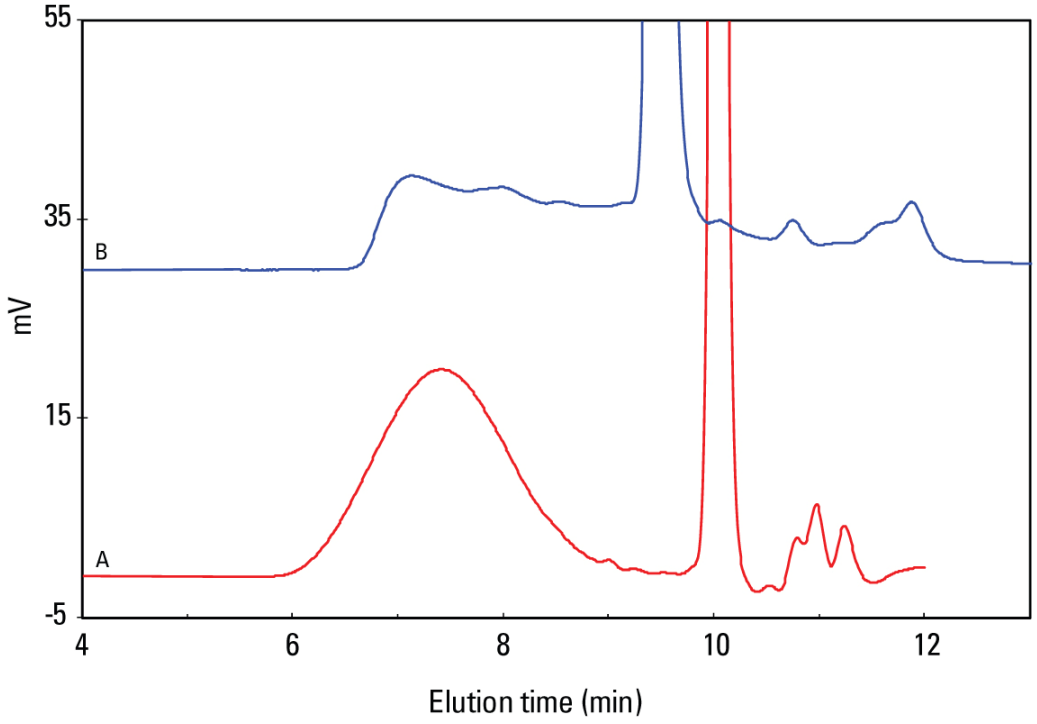
Mobile phase	Temperature (°C)	MW
A	60	20,000
B	40	19,000
C	25	19,000

In this case, differences in temperature (25°-60°C) did not affect the molecular mass measurements of PVP.

In general, the hydrophobicity of polymers increases with increasing temperature.

Comparison of analysis of PVP on small pore TSK-GEL columns

Figure 8: Comparison using TSKgel SuperMultiporePW-N and conventional TSK-GEL columns in series

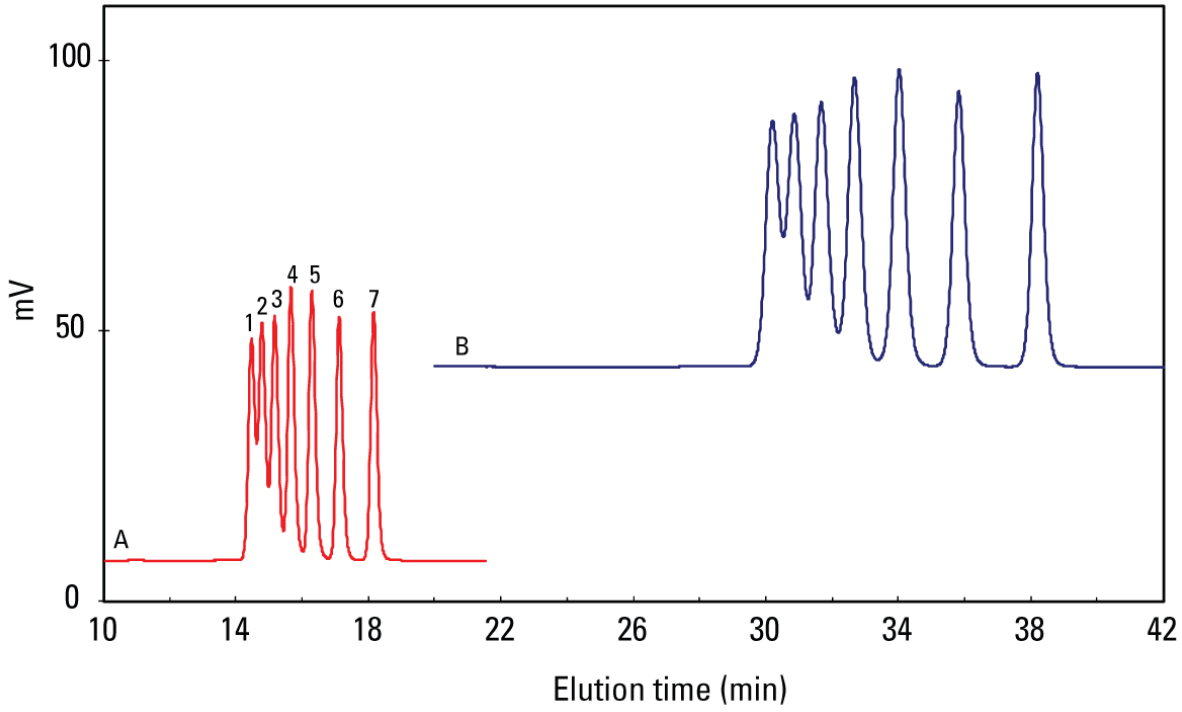


Columns:	A: TSKgel SuperMultiporePW-N, 6.0mm ID x 15cm x 2 B: TSKgel G3000PW$_{XL}$+G2500PW$_{XL}$, 6.0mm ID x 15cm x 2
Mobile phase:	100mmol/L $NaNO_3$
Flow rate:	0.60mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	20µL
Samples:	PVP (K-15)

Hydrophobic interaction causes partial adsorption of PVP-15 polymer on TSK-GEL G3000PW$_{XL}$ and G2500PW$_{XL}$ columns, while the absence of adsorption on the TSKgel SuperMultiporePW-N column suggests that the internal particle surface is more hydrophilic than the conventional columns.

Comparison of low molecular weight compounds

Figure 9: Fast separation of maltose oligomers using TSKgel SuperOligoPW and TSKgel G-Oligo-PW columns

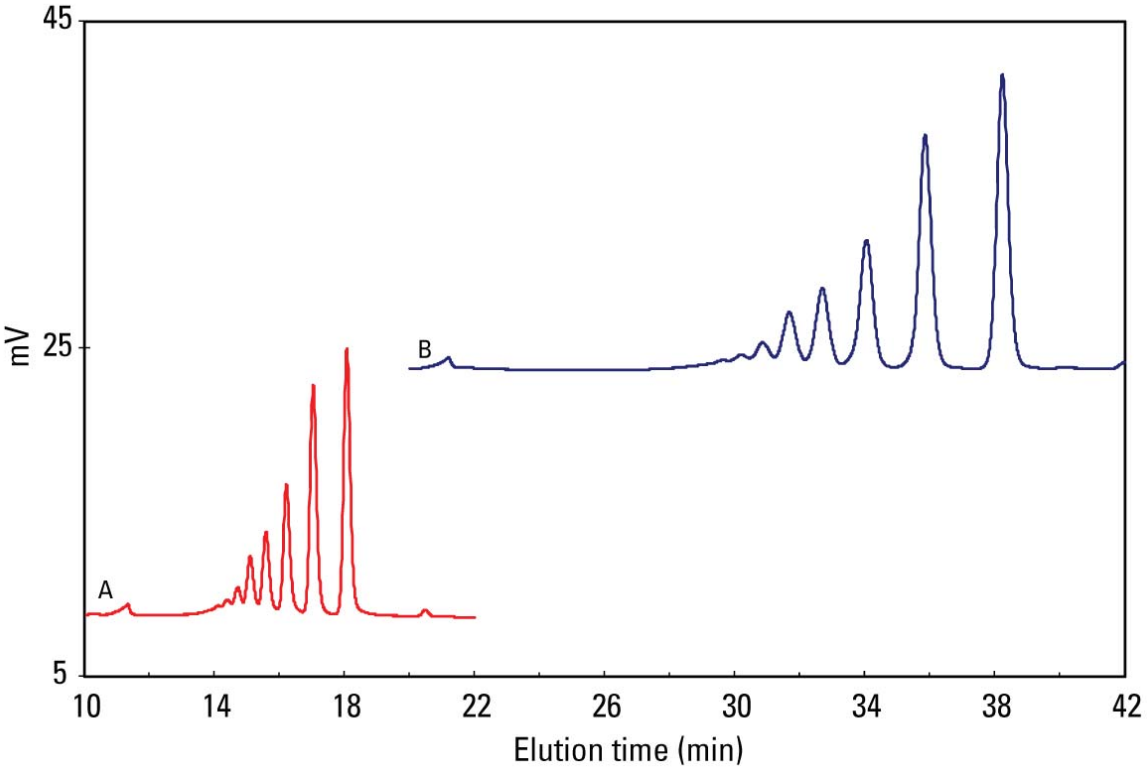


Columns: A: TSKgel SuperOligoPW, 6.0mm ID x 15cm x 4
B: TSKgel G-Oligo-PW, 7.8mm ID x 30cm x 4
Mobile phase: H_2O
Flow rate: A: 0.6mL/min B: 1.0mL/min
Detection: RI
Temperature: 40°C
Injection vol.: A: 10µL B: 50µL
Sample: 1.maltoheptose
2. maltohexose
3. maltopentose
4. maltotetraose
5. maltotriose
6. maltose
7. glucose

The separation was completed in less than ½ the analysis time on the TSKgel SuperOligoPW column.

Comparison of low molecular weight compounds

Figure 10: Fast separation of hydrolysate of maltopentose using a TSKgel SuperOligoPW and TSKgel G-Oligo-PW column



Columns:	A: TSKgel SuperOligoPW, 6.0mm ID x 15cm x 4 B: TSKgel G-Oligo-PW, 7.8mm ID x 30cm x 4
Mobile phase:	H_2O
Flow rate:	A: 0.6mL/min B: 1.0mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	A: 10µL B: 50µL
Sample:	hydrolysate of maltopentose

The separation was completed in less than ½ the analysis time on the TSKgel SuperOligoPW column.

Conclusions

- TOSOH recently introduced three grades of novel semi-micro columns for SEC of water-soluble polymers. TSK-GEL SuperMultiporePW columns are packed with spherical monodisperse polymethacrylate particles, each containing a wide range of pore sizes.
- To obtain optimum separations, the pore size should be selected by considering the molecular weights of the samples to be analyzed. Salt concentration and percent organic solvent should be optimized to improve accuracy and precision.
- TSK-GEL SuperMultiporePW columns exhibited good linearity of the calibration curves across a wide range of molecular mass for water soluble polymers in aqueous eluent.
- The concentration of salt and the percentage of water miscible organic solvent in the eluent affected the calculated molecular weight results by reducing hydrophobic interaction between sample and stationary phase. The new columns are less hydrophobic than the conventional columns.
- Various polymers can be analyzed on the TSK-GEL SuperMultiporePW and SuperOligoPW semi-micro columns. These novel columns provide reproducible and accurate results for the analysis of water-soluble polymers.