Which GPC Column First?

TSKgel
APPLICATION NOTE

Introduction

In the recently published 2nd edition of Modern Size Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography[1] it is stated that the debate about which column to place first when running a series of SEC columns is still open. This is despite most manufacturers' recommendation of installing the columns in order of decreasing pore size, the largest pore size column connected to the injector and the smallest to the detector. Scientists at Tosoh Bioscience tested the validity of this recommendation using 4.6mmID x 15cm TSKgel® GPC columns optimized for high speed analysis on the EcoSEC® GPC System.

In order to make the experiment as sensitive as possible to the effects of the order in which the sample encounters small versus large pores, two columns with 3 and 4µm particles that differ vastly in pore size and pore size distribution were chosen. The small pore column used was the TSKgel® SuperHZ1000 column with a mean pore size of 15Å and narrow pore size distribution. The large pore size column used was the TSKgel SuperMultiporeHZ-M with a mean pore size ca. of 800Å and a wide pore size distribution. It is reasoned that if there isn't any difference with these high resolution columns, there should not be any column arrangement differences for any two columns that have lower resolution.

When coupling the two columns in series, the TSKgel SuperHZ1000 column is either placed before or after the TSKgel SuperMultiporeHZ-M column. Using commercially available polymer standards, molecular weight averages were determined to see if the question of whether it is better to place the small pore column first or last could be answered.

Materials and Methods

Instrumentation:	EcoSEC GPC System (Tosoh)
Data Processing:	EcoSEC-WS (Tosoh)
Columns:	TSKgel SuperMultiporeHZ-M, 4µm, 4.6mm ID x 15cm TSKgel SuperHZ1000, 3µm, 4.6mm ID x 15cm
Mobile Phase:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temperature:	40ºC
Injection vol.:	10µL
Samples:	PStQuick MP-M polystyrene standards varying in MW from 500 to 706,000 Da (Tosoh Bioscience) Terathane® poly(tetramethylene ether glycol) T-1000 (Aldrich Chemical)
Concentration:	~1mg/mL in THF

Results

The objective of this study was to determine and compare the average molecular mass values of polymer standards using two columns in series when operating these columns in normal and reverse order. For the purpose of this study, the 'normal' order was arbitrarily defined as the one in which the largest pore size column was connected to the injector, followed by the smallest pore size column, which was connected to the detector.

Table 1 shows the M_w values of six polymer standards and their retention times and correlation coefficients for a cubic fit to the curve connecting the retention times, while *Table 2* shows retention times and calculated M_n, M_w, and M_z values for four distinct components in T-1000 PTMEG [poly(tetramethylene ether glycol)] polymer. The order in which the columns were installed in the EcoSEC GPC System is listed above each table. In all cases, column 1 is attached to the injector, while column 2 is connected to the detector.

Table1. Effect of Column Order on Retention Times and Weight Averaged Molecular Weight Values of Polystyrene Standards

1A: Normal column order

Column 1: TSKgel SuperMultiporeHZ-M Column 2: TSKgel SuperHZ1000		
M_w	Time (min)	Coefficient
706,000	9.45	A = 0.059
96,400	10.93	B = 0.192
5,970	12.70	C = 1.430
474	15.35	D = 4.493
370	15.70	
266	16.22	
Correlation coefficient = 0.994		

1B: Reverse column order

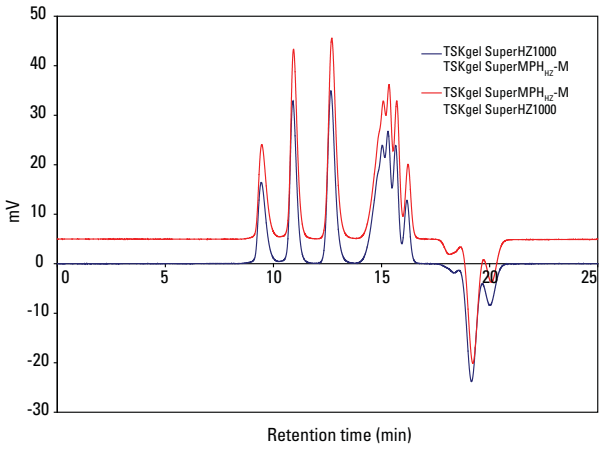
Column 1: TSKgel SuperHZ1000 Column 2: TSKgel SuperMultiporeHZ-M		
M_w	Time (min)	Coefficient
706,000	9.44	A = 0.059
96,400	10.90	B = 0.192
5,970	12.66	C = 1.419
474	15.30	D = 4.579
370	15.65	
266	16.17	
Correlation Coefficient = 0.994		

Cubic curve fit $At^3 + Bt^2 + Ct + D$

Discussion

Table 1 lists the coefficients of variation obtained by fitting the retention of six polymer standards to a cubic equation for each of the column arrangements. *Table1A* (normal order) represents data generated from columns arranged with the larger pore size packing followed by the smaller pore size packing. *Table 1B* (reverse order) has the opposite arrangement. Averages of three consecutive measurements are shown. Comparing the values of coefficients A, B, C and D it can be seen that there are no significant differences between the columns arranged in the normal or reverse order. An indication that the same conclusion holds true for oligomers in addition to polymers can also be seen from the data in *Table 1*. Visual proof that the order in which the columns are installed does not affect the chromatogram is shown in *Figure 1*.

Figure 1. TSKgel SuperMultiporeHZ-M and TSKgel SuperHZ1000 Run in Series



In a follow-up study, low molecular weight Terathane (PTMEG) polymers, which have higher polydispersity values, were used. The data presented in *Table 2* was obtained with Terathane T-1000, which showed four distinct oligomer peaks. Using calibration curves obtained with polystyrene standards, molecular mass averages M_n, M_w and M_z were calculated for each component. Again, a significant difference was not observed whether the columns were arranged in normal or reverse order.

Conclusions

The results of this limited study indicates that the order in which two GPC columns are arranged in series does not affect retention times, nor does it affect average molecular weight values.

Table 2. Effect of Column Order on Retention Times and Molecular Weight Averages for Terathane T-1000 Sample

2A: Normal column order

Column 1: TSKgel SuperMultiporeHZ-M Column 2: TSKgel SuperHZ1000				
Peak	Time (min)	M_n	M_w	M_z
1	13.20	2130	3320	5010
2	14.70	685	688	691
3	14.93	552	554	556
4	15.16	445	447	448

2B: Reverse column order

Column 1: TSKgel SuperHZ1000 Column 2: TSKgel SuperMultiporeHZ-M				
Peak	Time (min)	M_n	M_w	M_z
1	13.21	2180	3190	4750
2	14.71	680	684	688
3	14.86	546	548	550
4	15.10	443	445	447

[1]Striegel, A, et al (2009). Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography, Second Edition. John Wiley & Sons, Inc.

TOSOH

TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com