Reduce Oligosaccharide Analysis Time with TSKgel SuperOligoPW Size Exclusion Chromatography Columns Packed with Three Micron Particles

At the 2011 Pittsburgh Conference, Tosoh Corporation introduced a new line of high efficiency columns for the analysis of water-soluble polymers by gel filtration chromatography. The new product line consists of four columns, of which three contain particles that have been designed with the multi-pore particle synthesis technology which Tosoh scientists pioneered in the mid '90s. The fourth column, TSKgel SuperOligoPW, the topic of this application note, was developed to reduce the analysis time for oligosaccharides and other oligomers with molecular masses in the range of 100 and 3000. Currently, the TSKgel G-Oligo-PW column, which contains spherical 7µm particles packed in a 7.8mm ID x 30cm column, is the preferred column for size analysis of neutral oligosaccharides by gel filtration chromatography. This application note will demonstrate that analysis times of oligosaccharide separations can be reduced by 50%, which in turn can result in lowering cost, improving turnaround time, offering better service, or any combination of these factors.

The characteristics of the TSKgel SuperOligoPW column, together with those of the TSKgel SuperMultiporePW columns, are shown in *Table 1*.

As shown in *Table 1*, the TSKgel SuperOligoPW column is packed with monodisperse particles. The scanning electron micrograph (SEM) in *Figure 1* illustrates the narrow distribution of particle sizes, which helps to reduce the pressure drop across the column.

While a moderate column back pressure helps to reduce wear and tear on the HPLC system, lowering column back pressure is particularly important when working with multiple columns coupled in series, such as shown in the accompanying applications.

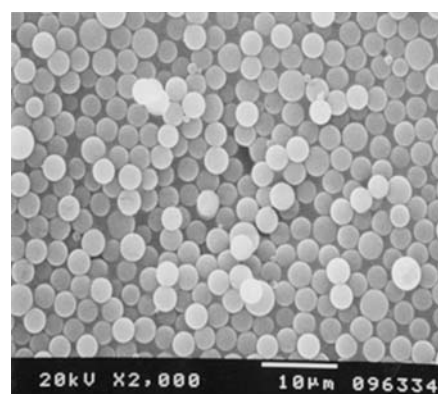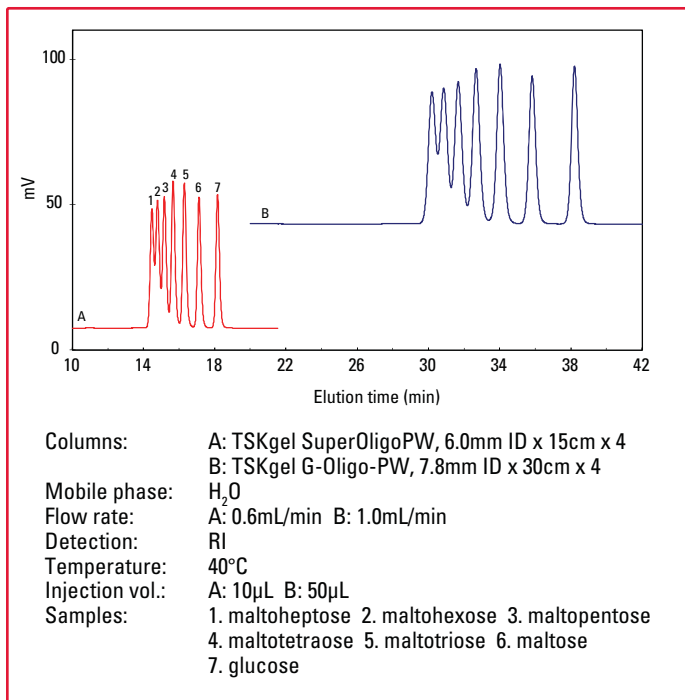**Figure 1.** Scanning Electron Micrograph (SEM) of particles contained in TSKgel SuperOligoPW columns

Table 1. TSKgel SuperOligoPW and SuperMultiporePW gel filtration columns

	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$_2$O)	4,000 - 8,000	100,000 - 150,000	600,000 - 1,500,000	–
Range of applicable MW (PEO,PEG/H$_2$O)	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

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Figure 2. Analysis of a maltodextrin sample on TSKgel SuperOligoPW and 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
Samples:	1. maltoheptose 2. maltohexose 3. maltopentose
	4. maltotetraose 5. maltotriose 6. maltose
	7. glucose

Figure 3. Calibration curves for TSKgel SuperOligoPW and SuperMultiporePW columns

Columns:	6.0mm ID x 15cm
	TSKgel SuperOligoPW
	TSKgel SuperMultiporePW-N
	TSKgel SuperMultiporePW-M
	TSKgel SuperMultiporePW-N
Mobile phase:	H_2O
Flow rate:	0.6mL/min
Detection:	RI
Temperature:	25°C
Sample:	PEO, PEG and EG

As is the case in all modes of HPLC, reducing the particle size by a factor of two (in this case from 7 to 3µm) either results in (1) the same analysis time but with much better resolution when using columns of the same length, or in (2) the same resolution but at least a 50% reduction in analysis time if the length of the smaller particle size column is half of that of the larger particle size column. This last benefit is illustrated in *Figure 2*, which shows that for a maltodextrin sample, with glucose units varying in length from maltoheptose to glucose, approximately the same resolution can be achieved in less than half the time on the 15cm TSKgel SuperOligoPW column when compared to the conventional 30cm TSKgel G-Oligo-PW column.

Figure 3 shows the calibration curves for each of the new TSKgel PW-type columns for polyethylene oxide (PEO), polyethylene glycol (PEG) and ethylene glycol (EG) standards. In contrast to the three TSKgel SuperMultiporePW columns, the calibration curve for the TSKgel SuperOligoPW column has a limited linear range that makes the column most suitable for oligosaccharides and other water-soluble polymers from a degree of polymerization of 1 to a molar mass of about 3000.

A comparative analysis of maltopentose hydrolysate is shown in *Figure 4*. Of each column type, four columns are coupled in series. It is clear that the shorter TSKgel SuperOligoPW columns allow for a substantial reduction in analysis time while resolution on this column set is very similar to that on four TSKgel G-Oligo-PW columns. Like the results shown in *Figure 2*, a 10µL injection on the four 15cm columns results in similar peak heights as obtained from a 50µL injection on the set of four 30cm columns. Part of this is due to smaller particle size, which leads to higher efficiency and thus less band broadening, but the shorter column length and the use of a narrower column diameter also play a role. The relative contributions of each of these band broadening effects have been calculated in *Table 2*.
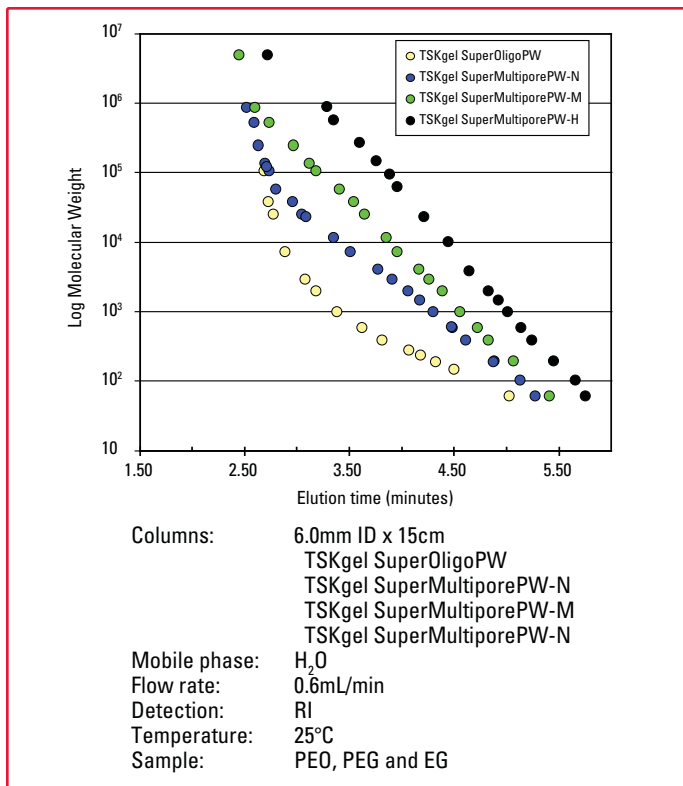
Figure 4. Analysis of maltopentose hydrolysate on TSKgel SuperOligoPW and G-Oligo-PW columns

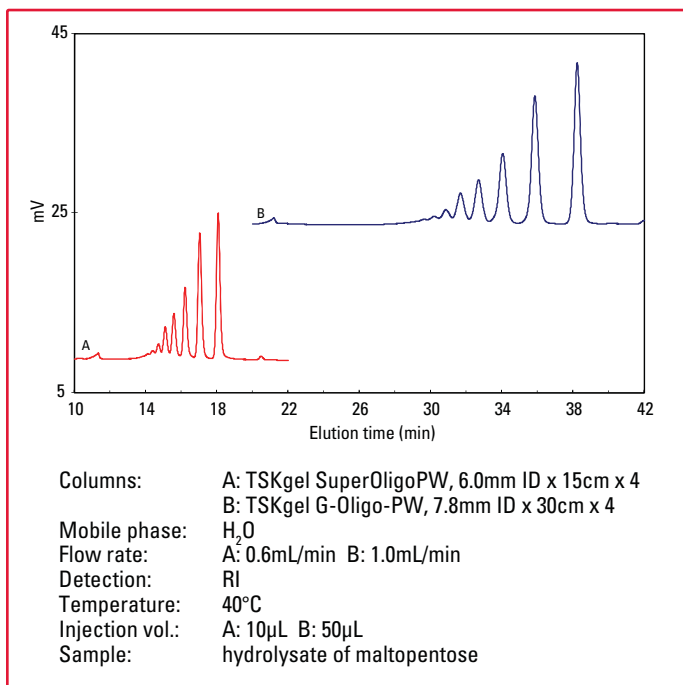| Columns: | A: TSKgel SuperOligoPW, 6.0mm ID x 15cm x 4 |
	B: TSKgel G-Oligo-PW, 7.8mm ID x 30cm x 4
Mobile phase:	H_2O
Flow rate:	A: 0.6mL/min B: 1.0mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	A: 10µL B: 50µL
Sample:	hydrolysate of maltopentose

Table 2. Relative importance of individual contributions to band broadening

Column size (mm ID x cm)	6.0 x 15	7.8 x 15	7.8 x 15	7.8 x 30
Column volume (μL)	4,239	7,164	7,164	14,328
Column porosity	0.80	0.80	0.80	0.80
Unretained volume (μL)	3,391	5,731	5,731	11,462
Efficiency (N)	16,000	16,000	14,000	14,000
Peak volume (μL)	107	181	194	387
Relative peak height	100	59	55	28

Notes

To calculate the relative importance of various contributions to peak height, *Table 2* shows two additional column dimensions as intermediate cases. The peak height of the 6mm ID x 15cm TSKgel SuperOligoPW column was arbitrarily selected as 100%. If we assume that a 7.8mm ID x 15cm column packed with the same 3μm particles would again have 16,000 theoretical plates, the signal would be 41% lower simply due to the larger internal diameter of the column. If the number of plates is reduced to 14,000 the peak height drops another 4%, while a 30cm column of 7.8mm ID and 14,000 plates, representing the conventional TSKgel G-Oligo-PW column, results in a further reduction of the peak height, down to 28% of the peak height of the new column. In other words, a 40μL injection, rather than a 50μL injection, would have resulted in the same peak heights at the same signal to noise ratio on both columns.

Conclusion

A new column for the analysis of oligosaccharides by size exclusion chromatography, TSKgel SuperOligoPW, provides the same resolution in half the analysis time and with a 4 to 5-fold improvement in sensitivity compared to the conventional TSKgel G-Oligo-PW column. This advancement was made possible by packing 3μm monodisperse spherical particles in 15cm columns of 6mm internal diameter. Applications demonstrated the expected benefit of the new column.

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