High-Throughput SEC of Water Soluble Polymers Using TSKgel SuperAW Columns

TSKgel
APPLICATION NOTE

Abstract

Recently, Tosoh has commercialized a more efficient, smaller particle of their hydrophilic polymethacrylate-based materials used widely for SEC of water soluble polymers. The TSKgel SuperAW column series is best used for high throughput analysis of water or polar organic soluble polymers.

Introduction

The need for fast analysis with reduced solvent and sample consumption has become a dominant force in HPLC column design. The TSKgel SuperAW series offers a range of particle sizes from 4µm to 9µm dependant on pore size, compared to 6µm to 13µm used in conventional polymer gel filtration chromatography (GFC). The smaller particles provide equivalent resolution in half the time of conventional columns. The highly cross-linked methacrylate backbone can withstand a range of solvents and solvent replacements from 100% water to 100% polar organic solvent systems without loss of performance.

Experimental Conditions

Comparison tests were run using a 4µm TSKgel SuperAW2500 column versus a conventional 6µm TSKgel G2500 PW_{XL}. Flow rates were adjusted to ensure a constant linear velocity. Similarly, sample volume was proportionally adjusted to avoid volume overload on the narrower bore columns. Solvent compatibility tests were assessed by measuring plate height after a switch from water to organic and back to water. The substitution used a flow rate of 0.6mL/min for a minimum of 5 hours. After substitution, the organic remained stagnant for a minimum of 14 hours. Water replaced the organic at a flow rate of 0.6mL/min for a minimum of 5 hours prior to measuring efficiency with an ethylene glycol standard.

Results

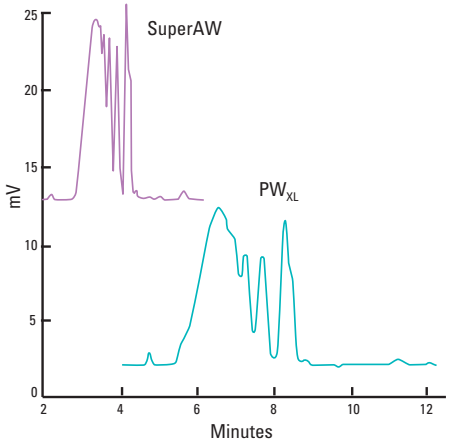
The link between efficiency and operational variables in chromatography was first described by van Deemter et al. in the 1950's and later refined by Giddings and, more recently, Knox. Column efficiency equations describe the various contributions to band broadening that take place when sample components travel through the column. The processes include (1) longitudinal diffusion, which plays a negligible role in practical HPLC, (2) dispersion of the sample band due to the velocity profile between particles, (3) dispersion due to the presence of the packed bed (often referred to as Eddy diffusion), which forces sample bands to go around the particles, and dispersion from resistance to (4) mobile phase and (5) stationary phase mass-transfer inside the particle. Except for longitudinal diffusion, terms (2) - (5) are all affected by particle size.

Smaller particle size columns have narrower flow paths between particles and the mean velocity in each flow path is lower, which results in less dispersion. Similarly, Eddy diffusion is reduced because of the shorter distance a solute has to travel to get around a particle. The smaller particle diameter also results in less time to traverse the stagnant mobile phase volume in the pores, which in SEC can be looked at as the stationary phase. The end result is that the sum of all dispersion processes leads to narrower bands, which the advantage, of course, is paid for at the price of higher column back pressure.

Researchers are given a choice when diffusion is minimized by smaller sized particles; either utilize the extra resolving power or shorten the analysis time by reducing column length. *Figure 1* shows the latter approach. Resolution of a dextran T-40 standard on the TSKgel SuperAW column is the same as for the larger 6µm TSKgel PW_{XL} column, but this result was obtained in half the time.

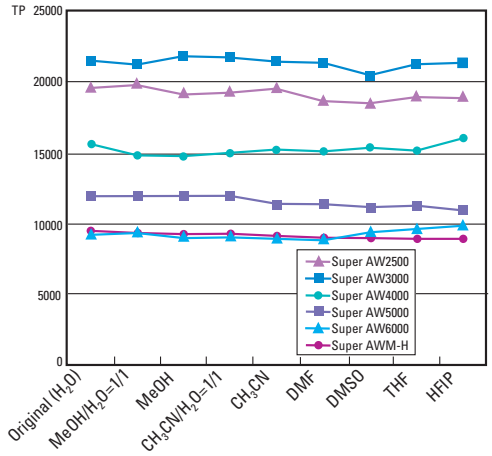
The shorter column length of a TSKgel SuperAW column, combined with a narrow bore, reduces solvent consumption and disposal cost. *Figure 2* shows the durability of the column in a variety of aqueous to polar organic mobile phases. The high degree of cross linking results in a stable particle that resist shrinking or swelling with repeated solvent changes.

Figure 1. Comparison of TSKgel SuperAW2500 and TSKgel G2500PW_{XL}



Column:	TSKgel SuperAW2500 (6.0mm ID x 15cm) TSKgel G2500PW_{XL} (7.8mm ID x 30cm)
Eluent:	Water
Flow rate:	0.6mL/min (TSKgel SuperAW2500) 1.0mL/min (TSKgel G2500PW_{XL})
Temperature:	25°C
Detection:	RI
Sample:	dextran T-40 hydrolysate

Figure 2. Solvent Compatiblity of TSKgel SuperAW Column Series



Column:	TSKgel SuperAW Series (6.0mm ID x 15cm)
Eluent:	Water
Flow rate:	0.6mL/min
Temperature:	25°C
Detection:	RI
Sample:	ethylene glycol
Inj. volume:	5µL (2.5g/L)

Conclusions

The TSKgel SuperAW columns demonstrate excellent resolution in less time than conventional polymeric SEC columns. TSKgel SuperAW columns provide consistent results independent of the aqueous or polar organic mobile phase chosen.

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