Stability of TSKgel SuperSW3000 Columns for Analyzing Proteins by SEC

TSKgel
APPLICATION NOTE

Abstract

TSKgel SuperSW3000 columns are stable under typical SEC conditions used for the analysis of proteins. High column efficiency is maintained by protecting the analytical column with a short guard column and by periodic guard column replacement.

Introduction

In SEC, resolution can be improved only by increasing the number of theoretical plates or by increasing pore volume. After maximizing pore volume, Tosoh researchers have most recently focused on improving the efficiency of silica-based SEC columns by reducing particle size from 10 to 5µm[1,2] The latest generation columns, TSKgel SuperSW, contain spherical, 4µm particles[3]. Similar to the popular TSKgel SW_{XL} and SW columns, the TSKgel SuperSW2000 and SuperSW3000 columns are bonded with a glycol ether–type bonded phase, resembling the well-known diol functional groups[1].

Although much is known about the stability of reversed phase columns[4], few authors, with the exception of Stout et al.[5], have studied the lifetime of size exclusion columns.

This study provides an initial investigation concerning the stability of the TSKgel SuperSW3000 column under standard operating conditions.

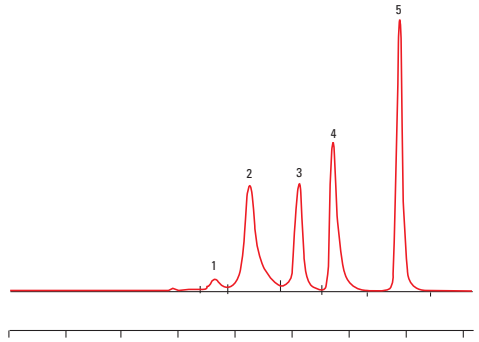
Experimental Conditions

Stability studies were performed with a 4.6mm ID x 30cm stainless steel TSKgel SuperSW3000 column, packed with spherical 4-µm particles containing 250Å pores. The experiment was run by injecting a standard mixture of proteins every 20 minutes and measuring column efficiency after every 5–10 injections during the first 500 injections. After 500 injections, efficiency was checked roughly every 100 injections. Fresh mobile phase was made throughout the duration of the study.

Protein standards and buffer reagents were obtained from Sigma (St. Louis, MO). The void volume marker p-aminobenzoic acid (PABA) was obtained from Eastman Kodak (Rochester, NY). Standard HPLC equipment was used to perform all experiments.

The analytical column was protected by a 4.6mm ID x 3.5cm TSKgel SuperSW guard column, filled with SuperSW3000 packing material at all times. A total of seven guard columns were used during the course of this 4000-injection study. The frequency of replacement was on an as-needed basis, determined by a drop in efficiency or a sudden increase in back pressure.

Figure 1. Protein test mixture to assess column stability.

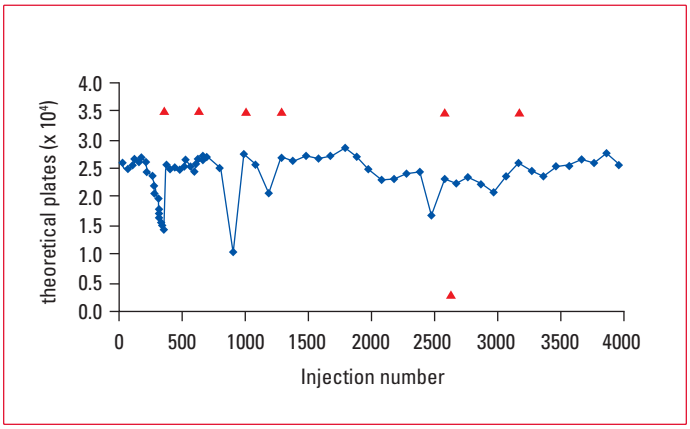


Column:	TSKgel SuperSW3000, 4.6mm ID x 30cm
Guard column:	TSKgel SuperSW3000, 4.6mm ID x 3.5cm
Injection:	5µL
Eluent:	0.1M phosphate buffer, 0.1M Na_2SO_4, 0.05% NaN_3
Flow:	0.35mL/min
Temperature:	ambient
Detection:	280nm, 1µL cell volume, 0.2 second response time
Peaks:	1 = impurity 2 = g-globulin (156 kDa, 1mg/mL) 3 = ovalbumin (43 kDa,1mg/mL) 4 = cytochrome C (12.4 kDa, 0.5mg/mL) 5 = PABA (137 Da, 0.01mg/mL) in mobile phase

Results

A sample chromatogram at the start of the experiment is shown in *Figure 1*. The globular proteins γ-globulin, ovalbumin and cytochrome C are well separated from each other and from p-aminobenzoic acid, which was used as the efficiency marker. Efficiency of the guard column plus analytical column was determined at selected intervals during the more than 4000 injections of this experiment. *Figure 2* shows how the number of theoretical plates varied as a function of the number of injections. An average efficiency of more than 24,000 plates ±12% was achieved throughout the study. Note that efficiency declined several times during the course of the study, as indicated by the triangular symbols in *Figure 2*. In all cases, the decline in efficiency was reversed by replacing the guard column with a fresh one. Given that it was outside the scope of this study, we did not investigate whether the drop in efficiency was related to a void or channel formation, uneven distribution of the sample over the top of the (guard) column, or buildup of debris from the pump, injector, insoluble sample, or mobile phase components. When excluding the runs prior to the guard column changes from the data set, the average efficiency over the duration of the experiment was 26,000 plates ±4.6%. This improvement indicates the importance of changing the guard column on a regular and frequent basis to continue to achieve optimum column performance. Obviously, the longevity of any column depends on many factors, including mobile phase, sample conditions and other system factors. Although a lifetime of 4000 injections with a TSKgel SuperSW column is not guaranteed, a lifetime of 700–1000 injections has been reported without the use of a guard column[6].

Figure 2. Variation of column efficiency with injection number. Column, guard column and other conditions as in Figure 1. Data points indicate sample injection to determine column efficiency and other parameters. Red triangles indicate guard column replacement due to either a drop in efficiency or an increase in pressure drop.



Conclusion

The TSKgel SuperSW3000 column maintains its performance and longevity if the proper preventative maintenance of changing the guard column, when needed, is performed .

References:
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