



Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns

Application Note

Authors

Greg Saunders and Umbreen Ahmed
Agilent Technologies, Inc.

Introduction

Obtaining good quality size exclusion data for a protein sample requires a column with a suitable molecular weight resolving range, such as ProSEC 300S. The easiest way to probe the resolving range of an SEC column is to analyze a series of chemically and structurally homologous polymers differing only in the molecular dimensions. In the case of a column for use in water or buffers such as those used in protein analysis, polyethylene glycol/oxide standards (which differ only in the nature of the chain end groups) are excellent candidates.

To investigate the molecular weight resolving range of the ProSEC 300S column a series of polyethylene glycol/oxide standards were analyzed and their elution order plotted as a function of molecular weight.



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Methods and Materials

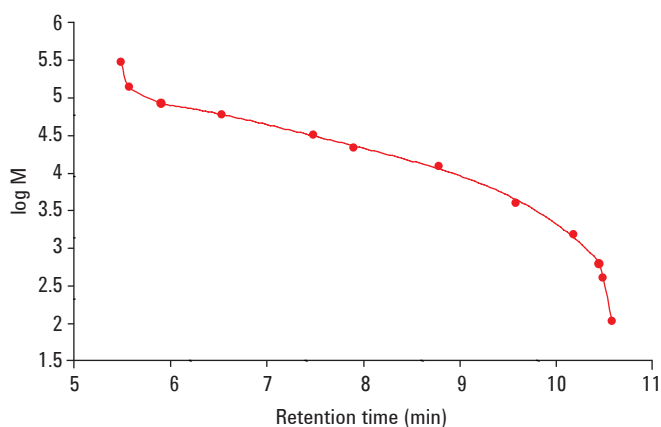
Conditions

Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent:	0.3M: 50mM KH ₂ PO ₄ -K ₂ HPO ₄ , pH 6.8, containing 0.3M NaCl
Flow Rate:	1.0 mL/min
Inj Vol:	20 µL
Sample Conc:	4 mg/mL
Temp:	25 °C
Detection:	UV at 280 nm

Conclusion

The wide resolving range of the ProSEC 300S column is suitable for the analysis of a wide variety of proteins on the basis of their size in solution and molecular weight. ProSEC 300S columns contain a packing with a surface modified for compatibility with proteins, ensuring that true size exclusion is obtained with minimal unwanted interaction effects. The pore size of the packing has been specifically selected to allow the analysis of a wide range of small to medium-sized proteins.

Results and Discussion



The calibration curve shows the relationship between the log of the molecular weight and the retention time, illustrating the wide resolving range of the ProSEC 300S column. The column resolves up to around 70,000 Daltons, which would equate to the molecular dimensions of a large sized globular protein.

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