Globular Proteins and the Calibration of Agilent ProSEC 300S Columns

Application Note

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Introduction

Protein analysis is of key interest in clinical research and medicinal chemistry. As part of this work, the isolation and purification of proteins is an important step. Many techniques have been developed to purify proteins and size exclusion chromatography (SEC), the separation of protein molecules on the basis of their size in solution, is one of the most versatile techniques. SEC also has the benefit of showing size heterogeneity of samples due to physical process such as aggregation, and can be an important analytical tool for studying protein behavior in solution.

Ensuring that the only separation obtained when analyzing proteins by SEC is based on size can be difficult, as protein molecules often contain hydrophobic and polar groups that can interact with the packing material in the column. To combat this, the characteristics of the column packing must be designed to minimize protein interactions. To illustrate the efficacy of the ProSEC 300S column a series of globular proteins was analyzed and their elution order plotted as a function of molecular weight.

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Methods and Materials

Table 1. Molecular weights of the proteins

Mw / Daltons	Protein
670,000	Thyroglobulin
150,000	γ-Globulin
66,430	Bovine serum albumin
44,287	Ovalbumin
29,000	Carbonic anhydrase
16,700	Myoglobulin
12,384	Cytochrome C
1,423	Bacitracin

Conditions

Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent:	0.3M: 50mM KH_2PO_4-K_2HPO_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

Results and Discussion

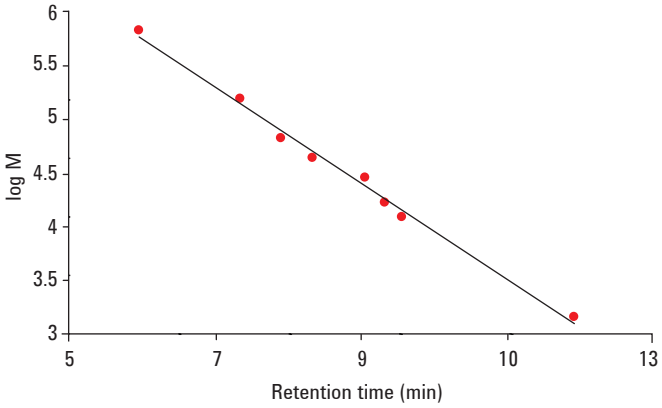

Figure 1. Calibration of the ProSEC 300S column using a selection of globular proteins

The calibration curve shows a linear relationship between the log of the molecular weight and the retention time, demonstrating that a pure size exclusion separation is taking place.

Conclusion

This note shows how a single ProSEC 300S column can analyze a selection of proteins on the basis of their size in solution and molecular weight, giving pure size exclusion for these materials. These columns have been specifically designed for the analysis of proteins by SEC. Using the ProSEC 300S column, protein interactions with the packing material that can occur in liquid chromatography are minimized, ensuring that a simple size-based separation is obtained.

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