Application Brief

Biopharma/Pharma

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GPC/SEC Analysis of Proteins From a Medium to High Molecular Mass Range

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Abstract

This application brief illustrates the GPC/SEC analysis of proteins using an aqueous buffer as eluent in combination with an Agilent Proteema column.

Introduction

Investigations about the aggregation and fragmentation of proteins are essential information in biopharmaceutical workflows. These data can be obtained by GPC/SEC analysis.[1]

The challenge of GPC/SEC analysis of proteins is to avoid undesired nonspecific interactions with the stationary phase. Various proteins can be analyzed using Agilent PROTEEMA columns by choosing the porosity according to the molar mass range in question.

Experimental

See Table 1.

Results and discussion

Robust and reliable GPC/SEC analysis of proteins was performed using a PROTEEMA column as stationary phase and aqueous phosphate buffer at pH = 6.6 and 0.5 M sodium chloride as eluent. For the analysis of medium to high molar mass proteins, a PROTEEMA 3 µm 300 Å column was employed. Figure 1 shows an overlay of the chromatograms of five proteins (thyroglobulin, gamma-globulin, albumin from bovine, albumin from chicken, and beta-lactoglobulin) ranging from 670,000 to 35,000 Da.

Table 1. Instrument and sample conditions.

	Conditions
Pump	Isocratic pump Flow rate: 1 mL/min Mobile phase: 34 mM phosphate buffer, pH 6.6, 0.5 M sodium chloride
Injection System	Autosampler Injection volume: 20 µL
Columns	PROTEEMA 3 µm 300 Å, 8 × 300 mm (p/n PRA0830033e2)
Temperature	23 °C
Sample Concentration	1 mg/mL
Detectors	Variable wavelength UV-Vis detector (VWD) at λ = 280 nm Refractive index (RI) detector
Software	Agilent WinGPC

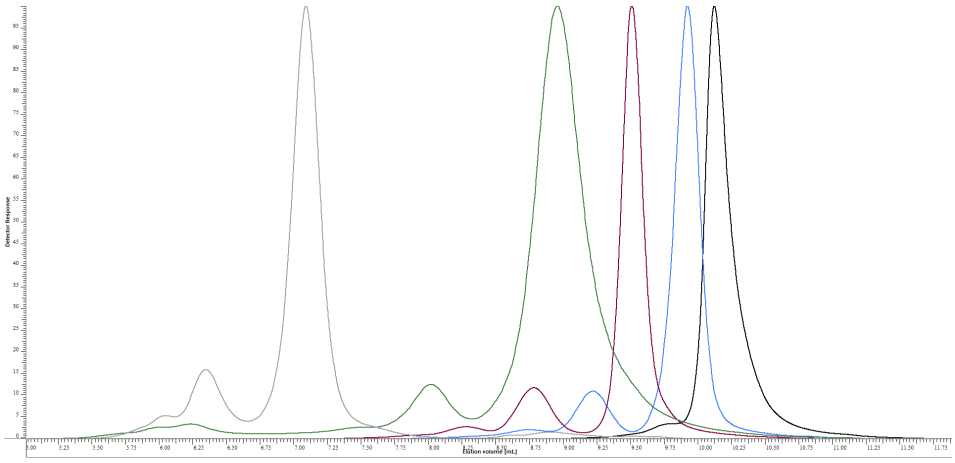

Figure 1. Overlay of UV at 280 nm traces (normalized detector response) for five proteins ranging from 670,000 to 35,000 Da using an Agilent PROTEEMA 3 µm 300 Å column.

Conclusion

PROTEEMA columns are suitable for GPC/SEC analysis of proteins in aqueous buffers. Proteins from medium to high molar mass can be separated according to their size on an Agilent PROTEEMA 300 Å column, which enables the detection of aggregates and fragments.

Reference

1. Hong, P.; Koza, S.; Bouvier, E. S. P. Size-Exclusion Chromatography for the Analysis of Protein Biotherapeutics and their Aggregates *J. Liq. Chromatogr. Relat. Technol.* **2012**, *35(20)*, 2923–2950.

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