Application Brief

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GPC/SEC Analysis of Proteins from Low to Medium Molecular Weight

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Abstract

This application brief illustrates the GPC/SEC analysis of proteins dissolved in an aqueous buffer solution using the Agilent PROTEEMA column as the stationary phase.

Introduction

Information about aggregation and fragmentation of proteins is key for many analytical investigations of biopharmaceuticals. This can be achieved 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 PROTEEMA columns by choosing the porosity according to the molecular weight range in question.

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.

Figure 1 shows an overlay of the chromatograms of five proteins (albumin, beta-lactoglobulin, myoglobin, cytochrome C, and aprotinin) and two additional small biomolecules (vitamin B12 and cytidine) ranging from 67,000 to 243 Da. The chromatography was performed on a PROTEEMA 3 µm 100 Å column, which allows good resolution in the low and medium molecular weight region.

Experimental

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 100 Å, 8 × 300 mm (p/n PRA0830031e2)
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

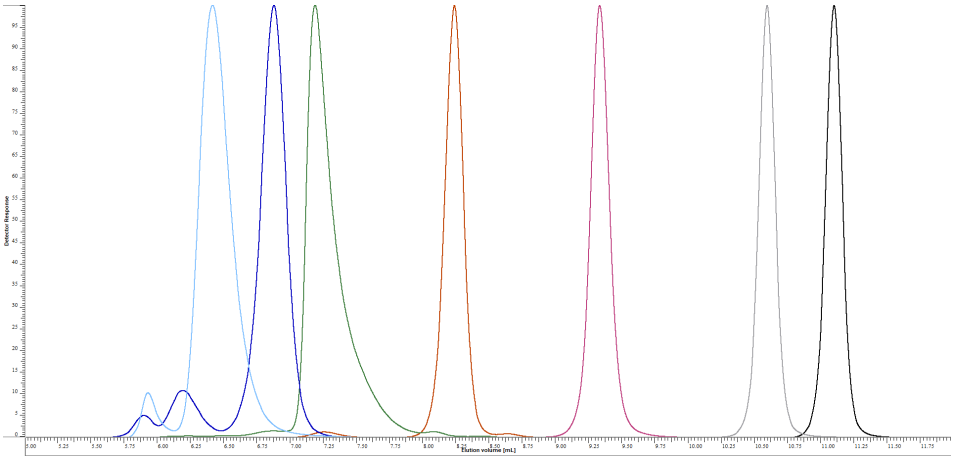

Figure 1. Overlay of UV at 280 nm traces (normalized detector response) for five proteins and two additional small biomolecules ranging from 67,000 to 243 Da using an Agilent PROTEEMA 3 µm 100 Å column.

Conclusion

Agilent PROTEEMA columns are suitable for GPC/SEC analysis of proteins in aqueous buffers. Proteins from low to medium molecular weight can be separated according to their size on a PROTEEMA 100 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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