Application Brief

Materials Testing & Research

Saccharide and Polysaccharide Analysis

High resolution in GPC/SEC with smaller particle size

Authors

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Abstract

Robust and reliable GPC/SEC analysis of a large variety of saccharides and polysaccharides can be performed with very high resolution using Agilent SUPREMA columns with 3 and 5 µm particles.

Introduction

Polysaccharides are linear or branched macromolecules composed of monosaccharides connected by glycosidic linkages. Polysaccharides occur widely in nature and have applications within areas such as the food, medical, or pharmaceutical industries.

Dextrans are used in various applications, where molecular weight is critical in determining the properties of the final product. Accurate determination of the molecular weight distribution is vital.

Low molar mass saccharides are also very common in food such as fruits, honey, and sweets. Examples for low molar mass sugars are mono- (glucose, fructose), di- (lactose, isomaltose, trehalose), and trisaccharides (maltotriose, isomaltotriose). The separation and identification of low molar mass polysaccharides is a challenge, as the compounds have the same chemical formula and only small differences in structure (e.g., disaccharides maltose, isomaltose, gentiobiose cellobiose and trehalose all have the chemical formula $C_{12}H_{22}O_{11}$).[1]

Experimental

Table 1. Instrument and sample conditions.

	Conditions
Pump	Isocratic pump Flow rate: 0.5 or 0.25 mL/min Mobile phase: H_2O, 0.05% sodium azide
Injection System	Autosampler Injection volume: 20 µL
Columns	SUPREMA 3 µm 100 Å, 8 × 300 mm (p/n SUA0830031e2) SUPREMA 5 µm 100 Å, 8 × 300 mm (p/n SUA0830051e2) SUPREMA low MW combination: SUPREMA 5 µm precolumn, 8 × 50 mm (p/n SUA080505) 3 × SUPREMA 5 µm 100 Å, 8 × 300 mm (p/n SUA0830051e2)
Temperature	23 or 80 °C
Sample Concentration	1 mg/mL
Calibration	Agilent calibration kit dextran (p/n PSS-DXTKIT)
Detectors	Refractive index (RI) detector
Software	Agilent WinGPC

Results and discussion

A high-resolution separation on the column is necessary for a precise analysis. This is particularly important when coupling GPC/SEC to mass spectrometry (MS), as the MS detector requires columns that provide higher resolution with smaller column volumes.

SUPREMA columns, with a reduced particle size of 3 and 5 µm, offer a significant improvement in performance compared to 10 µm materials, and provide additional resolution. This is of particular importance when analyzing oligomeric polysaccharides. Figure 1 shows a comparison of the resolution of a low molecular weight dextran with Mw = 1,260 Da obtained on a SUPREMA 10 µm 100 Å, SUPREMA 5 µm 100 Å, and SUPREMA 3 µm 100 Å column.

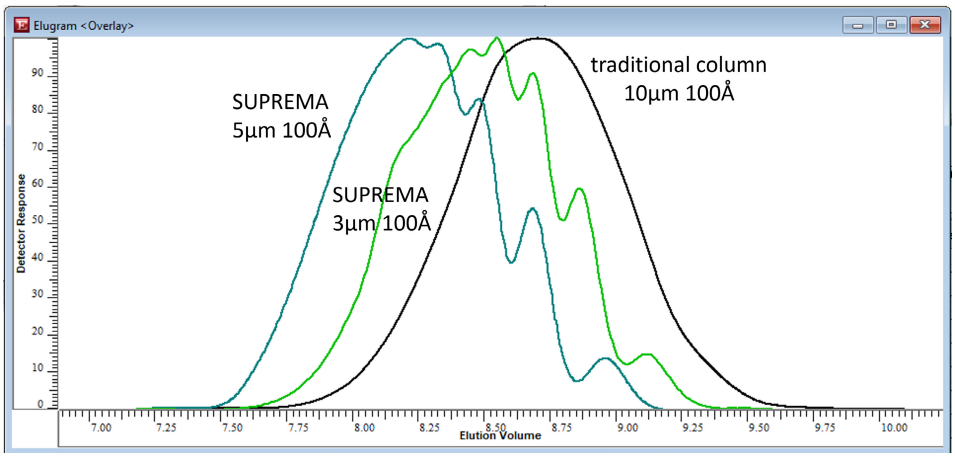

Figure 1. Overlay of elugrams showing RI trace, normalized detector response, and elution volume (mL) of dextran (Mw: 1,260 Da) at a flow rate of 0.5 mL/min in H_2O, 0.05% NaN_3 (red: typical 10 µm 100 Å column, dark green: Agilent SUPREMA 5 µm 100 Å, light green: Agilent SUPREMA 3 µm 100 Å))

The analysis of the same dextran sample (Mw: 1,260 Da) on a SUPREMA low MW combination, comprised of three SUPREMA 5 µm 100 Å columns, shows the increase in resolution when increasing column length while maintaining the same column type (see Figure 2). The oligomers in the low molecular weight region are well-resolved up to oligomer P10. The chromatogram of glucose is overlaid, as a reference.

Conclusion

Agilent SUPREMA columns with 3 and 5 µm particle sizes can be used for numerous neutral and anionic aqueous applications in the molecular weight area starting from about 100 Da. The reduced particle size of 3 or 5 µm leads to high-resolution separations, especially in the low molar mass range, which enables high-quality analysis.

Reference

1. Karlson, P. Kurzes Lehrbuch der Biochemie für Mediziner und Naturwissenschaftler, Georg Thieme Verlag Stuttgart, **1988**.

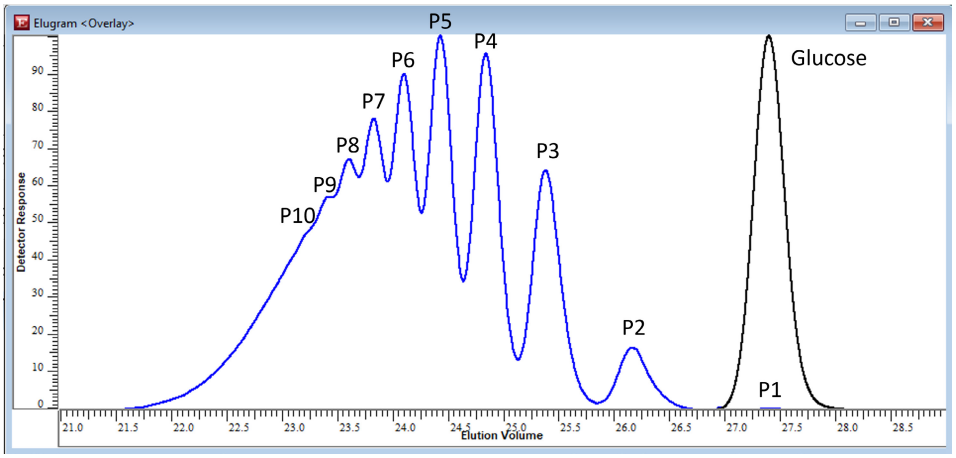

Figure 2. Overlay of elugrams showing RI trace, normalized detector response, and elution volume (mL) of dextran (Mw: 1,260 Da, blue) and glucose (mol. wt.: 180 Da, black) at a flow rate of 0.25 mL/min and 80 °C in H_2O, 0.05% NaN_3 with three Agilent SUPREMA 5 µm 100 Å columns.

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