Application Note

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Investigation of Iron Polysaccharide Complexes by GPC/SEC Using RI and UV Detection

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Abstract

Gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC), provides an easy and effective way to measure the molar mass distribution and the amount of free, unbound polysaccharide of iron polysaccharide complexes, if dual detection is used.

Introduction

Human bodies contain iron in red blood cells (as hemoglobin) or in muscle cells (as part of myoglobin). Both proteins are necessary for oxygen transport, and so iron is an essential nutrient. In cases of iron deficiency, complexes of a polysaccharide and iron are applied as drugs to enhance low iron levels. Suitable characterization of these complexes and their formulations is mandatory for regulatory reasons, quality control, and research.[1]

GPC/SEC provides an easy and effective way to measure the molar mass distributions of iron polysaccharide complexes. In this application note, iron polysaccharide complexes from different sources were analyzed on a GPC/SEC system with simultaneous UV/RI detection.

Experimental

Table 1. Instrument and sample conditions.

	Conditions
Pump	Isocratic pump Flow rate: 1 mL/min Mobile phase: H_2O, 0.01 M phosphate buffer (pH 7), 0.1 N $NaNO_3$
Injection System	Autosampler Variable injection volume
Columns	Agilent SUPREMA MW medium combination: Agilent SUPREMA 5 µm precolumn, 8 × 50 mm (p/n SUA080505) Agilent SUPREMA 5 µm 30 Å, 8 × 300 mm (p/n SUA0830053e1) 2 × Agilent SUPREMA 5 µm 1,000 Å, 8 × 300 mm (p/n SUA0830051e3)
Temperature	23 °C
Sample Concentration	– 2 g/L for dry material – 50 g/L for formulations
Calibration	Agilent ReadyCal-Kit Pullulan high (p/n PSS-PULKITR1H)
Detectors	Variable wavelength UV-Vis detector (VWD) at λ = 254 nm Refractive index (RI) detector
Software	Agilent WinGPC

Results and discussion

An advantage of this application is that the iron polysaccharide complex is selectively detected by the UV detector operated at 254 nm. The more universal RI detector reveals the complex, unbound polysaccharide and the typical, unavoidable system peaks.

Interestingly, this possibility of selective detection of the iron complex by UV is frequently ignored. Instead, evaluation of the more complex RI trace is performed in most studies.

However, for this application, the UV traces were used to measure the molar mass distributions, molar mass averages, and dispersities of the iron polysaccharide complexes. For an in-depth characterization of the unbound polysaccharide, the RI detector signal was used.

Figure 1 shows the overlay of the UV chromatograms for the four different samples A, B, C, and D. Samples A and B show nearly identical elution profiles and cannot be differentiated when only UV detection is applied. The UV detector also does not show any unbound polysaccharide due to missing chromophores. This fact allows easy data evaluation. All complexes reveal well-shaped, nearly Gaussian peak shapes, indicating that the high molar mass exclusion limit and the low molar mass separation limit of the SUPREMA column combination are not reached for any of the samples analyzed. This means that the SUPREMA column medium MW combination is ideal for this molar mass separation range.

Three of the four samples can be clearly differentiated based on their chromatograms and the resulting molar masses. However, samples A and B render identical elution profiles.

All UV signals can easily be evaluated, as potentially coeluting residual components are invisible for the UV detector at the selected wavelength.

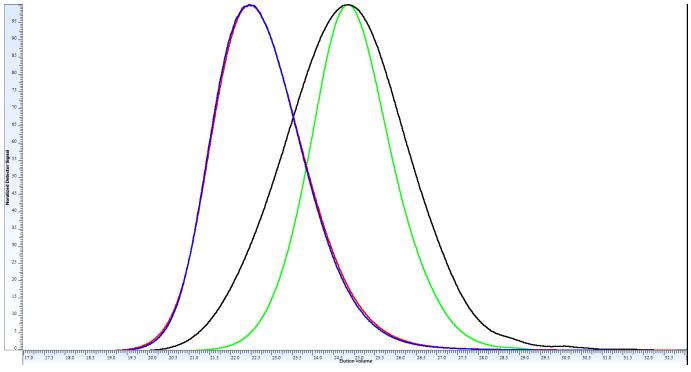

Figure 1. Normalized UV traces (UV at 254 nm) of four different iron polysaccharide samples: sample A (blue), sample B (red), sample C (black), and sample D (green).

By applying a 12-point calibration curve, established using ReadyCal-Kit pullulan high ranging up to approximately 1,200,000 g/mol, the relative molar mass distributions as well as the molar mass averages and the dispersities are derived. These results are summarized in Table 1.

Table 2. Molar mass averages and dispersities (Đ) of the pure iron polysaccharide complex, as derived from UV detection.

Sample	Mw (g/mol)	Mn (g/mol)	Đ
A	155,000	106,000	1.46
B	154,000	108,000	1.42
C	66,400	27,600	2.40
D	67,000	32,400	2.07

For the two samples A and B, which have identical elution profiles in the UV trace, differences can be found when reviewing the simultaneously measured RI signals (Figure 2). When comparing the RI traces, it becomes clear that sample A contains a significantly higher amount of the unbound polysaccharide. The RI detector signal reveals that sample A contains more unbound polysaccharide than sample B.

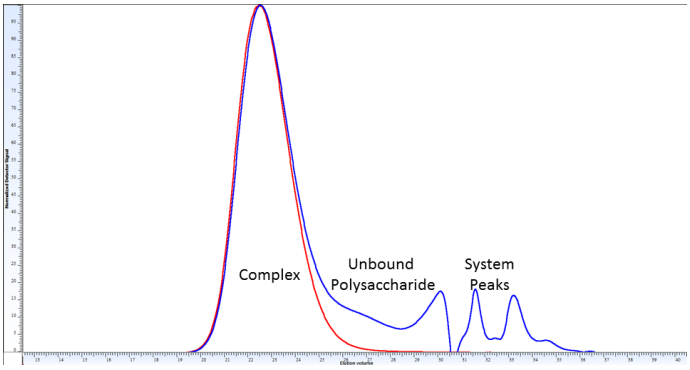

Figure 2. RI traces for samples A (blue) and B (red), which showed nearly identical UV signals.

Therefore, GPC/SEC with UV and RI detection not only allows the determination of the molar mass distribution of iron polysaccharide complexes, but simultaneously provides information on the amount of free, unbound polysaccharide, ensuring a more comprehensive characterization of the samples. This information is essential for quality control and enables optimization of the production processes.

Conclusion

Robust and reliable GPC/SEC separation of polysaccharide iron complexes is achieved with a set of Agilent SUPREMA columns as the stationary phase and an aqueous mobile phase composed of phosphate buffer and sodium chloride.

GPC/SEC with dual detection, composed of a UV-Vis and RI detector, allows comprehensive analysis of polysaccharide iron complexes. The whole sample is detected via RI, independent of whether it is free polysaccharide or bonded as an iron complex. In combination with UV detection, which only reveals polysaccharide iron complexes, RI enables the determination of the bonded and unbonded polysaccharide, besides conventional molar mass analysis.

Reference

1. Lu, R. *et al.* Efficacy and Safety of Polysaccharide Iron Complex Capsules Compared with Iron Sucrose in Hemodialysis Patients: Study Protocol For a Randomized, Open-Label, Positive Control, Multicenter Trial (IHOPE). *Trials* **2021**, *22(1)*, 691.

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