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

Application Note Pharmaceutical Analysis

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Dual-detection 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.

Introduction
Human bodies contains iron in the red blood cells (as hemoglobin) or in muscle cells (as part of myoglobin). Both proteins are necessary for oxygen transport. Thus, iron is an essential nutrient. In case 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 are mandatory for regulatory reasons, quality control and research.

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

System Requirements

	Conditions
Pump	PSS BioSECcurity quaternary pump • flow rate [mL/min]: 1.00 • mobile phase: aqueous, 0.01 M phosphate buffer (pH = 7)+ 0.1 N $NaNO_3$
Injection system	PSS BioSECcurity Autosampler • variable injection volume
Columns	• PSS SUPREMA precolumn (8*50mm) • PSS SUPREMA 5µ 30 Å + 1 000 Å (2x) (8*300mm each)
Injected mass	• 2 g/L for dry material, 50 g/L for formulations
Calibration	• PSS Pullulan ReadyCal Standards
Detectors	• BioSECcurity 1260 MWD at λ=254 nm • SECcurity 1260 RI
Software	PSS WinGPC UniChrom • optional for 21CFR11 compliance: Compliance Pack

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Procedure, Results & 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 detects the complex, unbound polysaccharide and the typical and 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 of the studies.

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

Figure 1 shows the overlay of the UV-chromatograms for the 4 different samples A, B, C and D. All complexes reveal well shaped nearly Gaussian peak shapes, indicating that neither the high molar mass exclusion limit nor the low molar mass separation limit of the PSS SUPREMA column combination is reached for any of the samples analyzed. This means that the PSS SUPREMA column 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 be easily evaluated as potentially co-eluting residual components are invisible for the UV-detector at the selected wavelength.

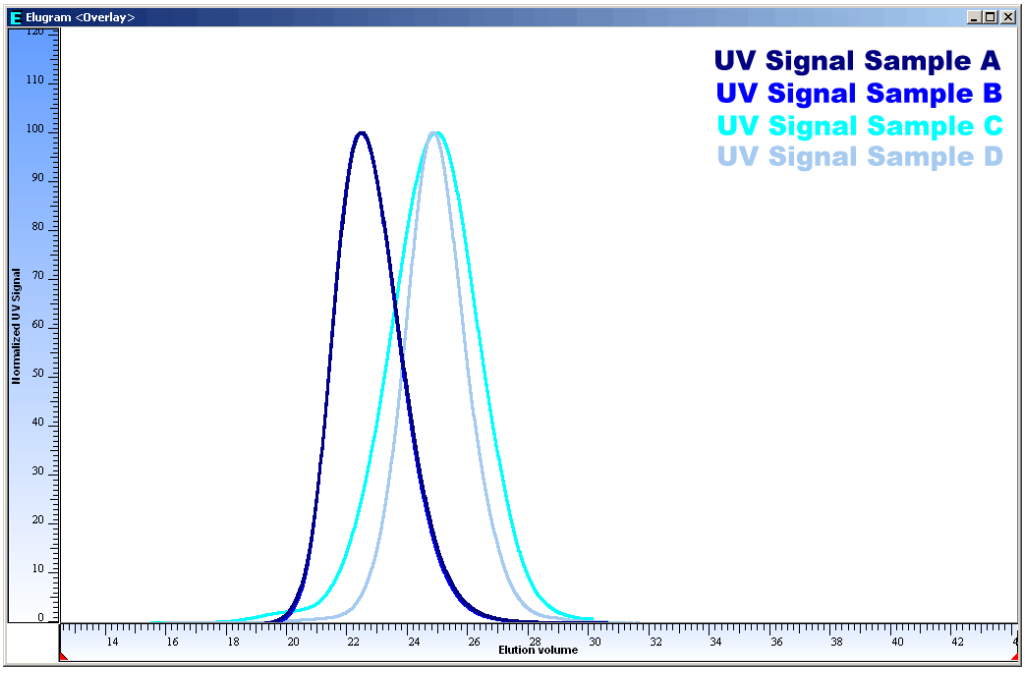

Fig. 1:
Normalized UV-traces of four different iron polysaccharide samples. Sample A and sample B show nearly identical elution profiles and cannot be differentiated when only UV-detection is applied. Furthermore the UV-detector does not show any unbound polysaccharide due to missing chromophores. This allows easy data evaluation.

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By applying a 12 point calibration curve, established using PSS pullulan standards ranging up to 1.2×10^6 Da, the relative molar mass distributions as well as the molar masses averages and the polydispersities are derived. These results are summarized in Table 1.

Table 1:
Molar mass averages and polydispersities (PDI) of the pure iron polysaccharide complex, as derived from UV-detection.

Sample	M_w/ Da	M_n/ Da	PDI
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 render identical elution profiles in the UV, differences can be found when reviewing the simultaneously measured RI-signals (conf. Figure 2). When comparing the RI-traces, it becomes clear that sample A contains a significantly higher amount of the unbound polysaccharide.

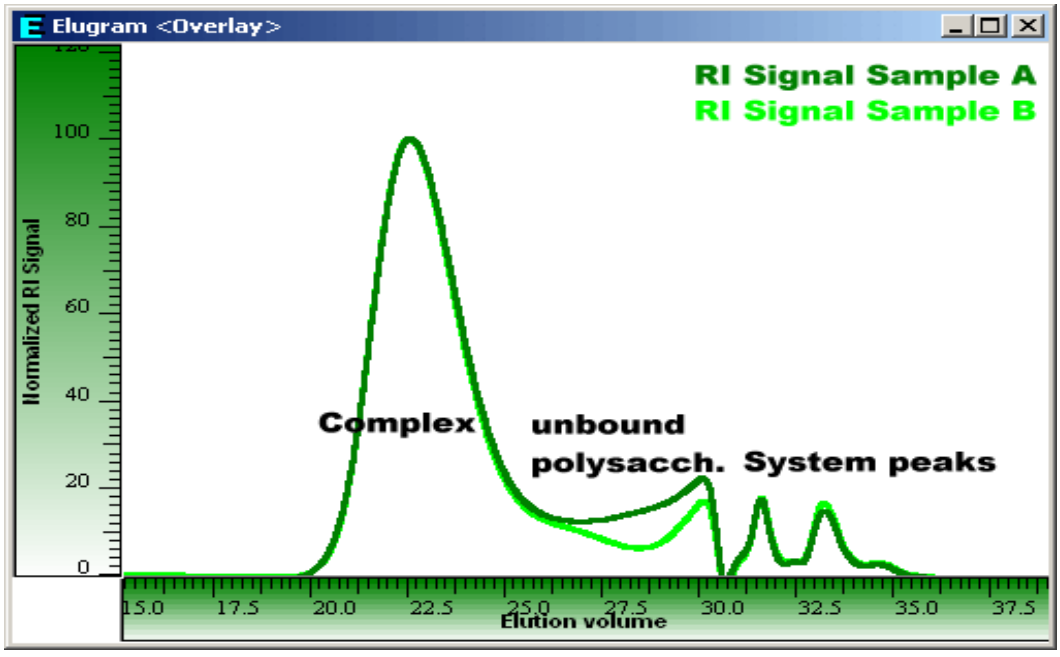

Fig. 2: RI-traces for the samples A and B, which showed nearly identical UV-signals. The RI-detector signal reveals that sample A contains more unbound polysaccharide than sample B.

We can therefore conclude that GPC/SEC with UV- and RI-detection not only allows the determination of the molar mass distribution of iron polysaccharide complexes, but at the same time 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 allows optimization of the production processes.

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