Analysis of Polysaccharides by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Application Note

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Introduction

Polysaccharides are complex polymers constructed from sugar units. There is a wide range of polysaccharides, many of which show large structural differences due to the manner in which they are synthesized. This is most commonly seen in the presence of branches on the polymer chains of some polysaccharides, which strongly influences properties such as solution viscosity. Pullulan polysaccharide is composed of maltotriose units in the polymer backbone, produced from starch by the action of a fungus. Pullulan has a linear structure, whereas in contrast dextran is a complex glucan with many differing components manufactured from sucrose by bacterial action that has a highly branched structure. Investigating the structure of polysaccharides is of interest for determining their properties in applications such as their use as food additives.

Gel permeation chromatography (GPC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many of the physical characteristics of these materials. GPC viscometry, employing a viscometer in combination with a differential refractive index detector, has the advantage of allowing the accurate determination of molecular weights for structurally complex polymers and co-polymers regardless of the their structure, via the Universal Calibration approach. GPC viscometry also reveals information about the solution viscosity of polymers, a property related to molecular size. Using this information, the branched structure of polymers can be investigated. This application note describes the analysis of two samples of polysaccharide by GPC viscometry, pullulan with a linear structure, and a highly branched dextran.

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Methods and Materials

Conditions

Samples:	Polysaccharides
Columns:	2 x Agilent PL aquagel-OH MIXED-M 8 µm, 300 x 7.5 mm (p/n PL1149-6801)
Injection Volume:	200 µL
Eluent:	0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Viscometer and DRI
Detector Temp:	All detectors set at 40 °C

The 390-MDS was chosen as part of the system as it is capable of multi-detector GPC in aqueous solvents and therefore allows the complex nature of these materials to be investigated.

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for a sample of pullulan polysaccharide. The material eluted as a broad peak.

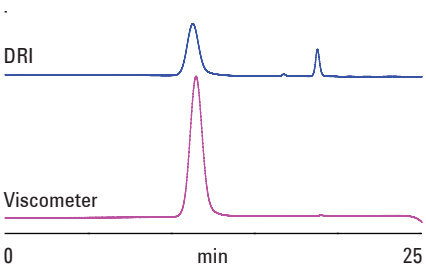

Figure 1. Overlaid multi-detector chromatogram for an example of pullulan polysaccharide

Figure 2 shows an overlay of the accurate molecular weight distributions of the two samples under investigation. As can be seen, they have very different molecular weight distributions.

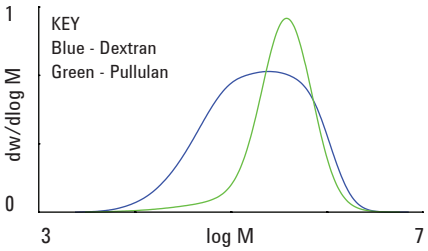

Figure 2. Overlaid multi-detector molecular weight distributions of two samples of polysaccharide

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight for the two samples. Compared to the pullulan, the dextran shows a marked shift of the Mark-Houwink plot to lower intrinsic viscosity values at any given molecular weight. This indicates that dextran is smaller in solution than pullulan across the molecular weight range, a result of the presence of branching on the dextran molecules. The dextran plot is complex and shows some changes in slope, indicating that the degree of branching varies across the range of molecular weight, as expected for a complex material.

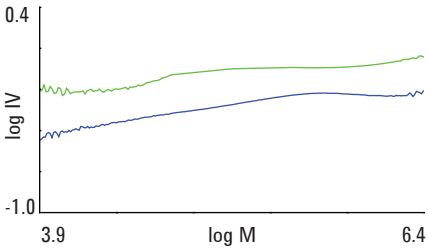

Figure 3. Overlaid Mark-Houwink plots for the two samples of polysaccharide

Conclusion

The data in this application note illustrates how multi-detector GPC employing the 390-MDS can be used to clearly see structural differences between pullulan and dextran with a highly branched structure.

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