Integrated Triple Detection for the Analysis of Pectins by SEC

Application Note

Food Testing & Agric

Authors

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Introduction

Pectins are a class of polysaccharide gum found naturally in fruits, such as apples, plums, grapes and cranberries. Structurally complex, pectins consist of 'smooth' and 'hairy' regions. The smooth regions are linear, partially methylated poly(D-galacturonic) acid, the hairy regions comprise alternating L-rhamnosyl and D-galacturonosyl residues containing L-arabinose and D-galactose branch points up to 20 residues long. As a result of this heterogenous nature, pectins adopt complex structures in solution. Applications of pectin are related to the formation of crosslinks through hydrogen bonding of the carboxylic acid groups, and include use as gelling agents, thickeners and water binders. Triple detection size exclusion chromatography employs a concentration detector, a viscometer and a light scattering detector to assess the molecular weight distribution and molecular structure of polymers without having to rely on column calibrations. This can be important when analyzing complex materials for which no structurally similar standards are available.

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Instrumentation

A sample of pectin was analyzed on the Agilent PL-GPC 50 Integrated GPC/SEC System. The instrument was operated at 30 °C and incorporated a refractive index detector, an Agilent PL-BV 400RT four capillary bridge viscometer and an Agilent dual angle light scattering detector (collecting scattered light at 15 ° and 90 °). Two Agilent PL aquagel-OH MIXED-H 8 μm columns were used for the analysis. These high performance columns offer excellent resolution over a very wide range of molecular weights, simplifying column selection and providing a versatile analytical system. The sample was prepared accurately in the eluent and filtered before injection through a 0.45 μm disposable filter. For the purpose of light scattering calculations, an average dn/dc value was used for the sample.

Conditions

Sample:	Pectin at 2 mg/mL
Columns:	2 × Agilent PL aquagel-OH MIXED-H 8 μm, 7.5 × 300 mm (p/n PL1149-6800)
Eluent:	0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 adjusted to pH 7
Flow Rate:	1.0 mL/min
Injection Volume:	200 μL
Sample Concentration:	Agilent EasiVial PEO Standards, 0.1-0.5 mg/mL
Polyacrylic Acid:	Approx 0.2 % w/v
Detection:	PL-GPC 50, RI, PL-BV 400RT, dual angle LSD

Results and Discussion

Figure 1 shows an overlay of the triple detector chromatograms for the pectin sample. The chromatograms from the refractive index and light scattering detectors were clearly multimodal, as expected for a structurally heterogeneous material.
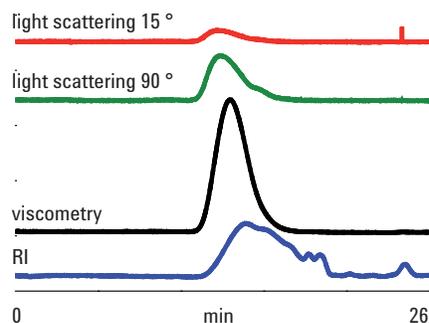

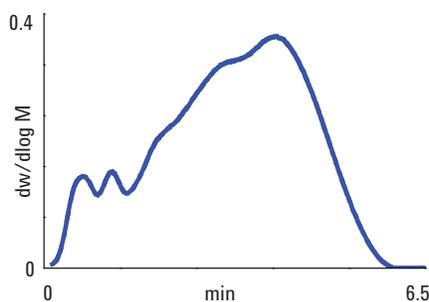Figure 2. Triple detector chromatograms of pectin (autoscaled)

Figure 2. Molecular weight distribution plot calculated for the pectin

From the viscometry and light scattering data, Mark-Houwink (log intrinsic viscosity versus log M) and conformation (log radius of gyration versus log M) plots were generated for the pectin, shown overlaid in Figure 3.
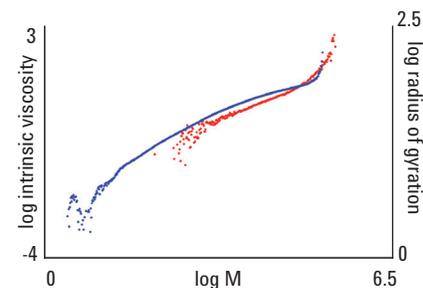

Figure 3. Overlaid Mark-Houwink and conformation plots for pectin

The Mark-Houwink and to some extent the conformation plots show curvature over the entire molecular weight distribution, indicating a change in molecular density as a function of molecular weight, resulting from a variation in the relative amounts of 'smooth' and 'hairy' regions.

Conclusion

The PL-GPC 50 Integrated GPC/SEC System is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PL aquagel-OH MIXED-H 8 μm columns, PL-BV 400RT viscometry and dual angle light scattering detectors, the PL-GPC 50 makes maximum use of triple detection for the accurate determination of molecular weights of structurally complex and commercially important polymers.

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