Polymer Laboratories

Analysis of Pectins by SEC with Triple Detection

Andrew Penrose and Greg Saunders
Polymer Laboratories, Inc.

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, and the hairy regions are 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 heterogeneous nature, pectins adopt complex structures in solution. Applications of pectin are related to the formation of cross-links 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.

Here, a sample of pectin was analyzed on the PL-GPC 50 integrated GPC system running at 30 °C fitted with a refractive index detector, a PL-BV 400 four capillary bridge viscometer, and a Precision Detectors' PD 2020 dual angle light-scattering detector (collecting scattered light at 15° and 90°). Two PL aquagel-OH MIXED 8 µm columns were used for the analysis with a 200 µl injection loop and a buffer solution of 0.2 M $NaNO_3$, 0.01M NaH_2PO_4, adjusted to pH 7, as the eluent. The sample was prepared accurately at nominally 2 mg/mL in the eluent and filtered before injection through a 0.45-µm disposable filter. For the purposes of light-scattering calculations, an average dn–dc value was used for the sample.

Figure 1 shows an overlay of the triple-detector chromatograms for the pectin sample. The chromatograms obtained on the refractive index and light-scattering detectors were clearly multimodal, as expected for a structurally heterogeneous material. Figure 2 shows the molecular weight distribution 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, as shown overlaid in Figure 3. The Mark-Houwink plot, and to some extent the conformation plot, shows 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 the smooth and hairy regions. This technical bulletin demonstrates how the new PL-GPC 50 can be used for the analysis of structurally complex but commercially important materials by multidetector GPC.

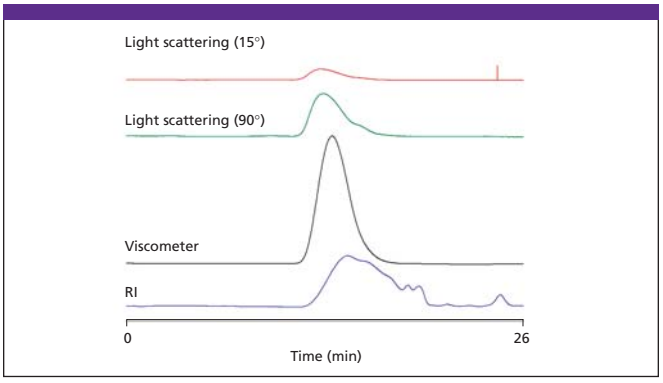

Figure 1: (Blue) Triple detection chromatogram of the pectin sample, refractive index, (black) viscometer, (red) light scattering 15°, (green) light scattering 90° (the chromatograms have been autoscaled).

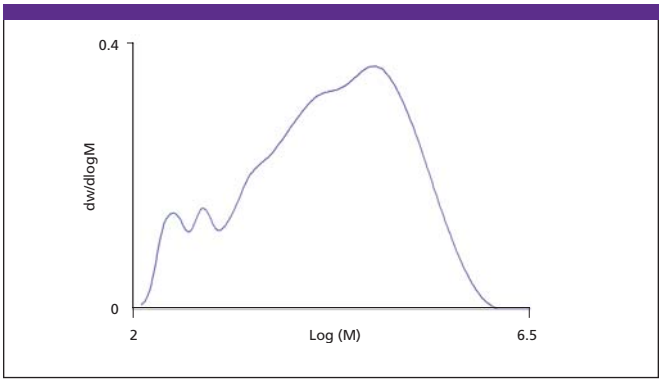

Figure 2: Molecular weight distribution for the pectin sample.

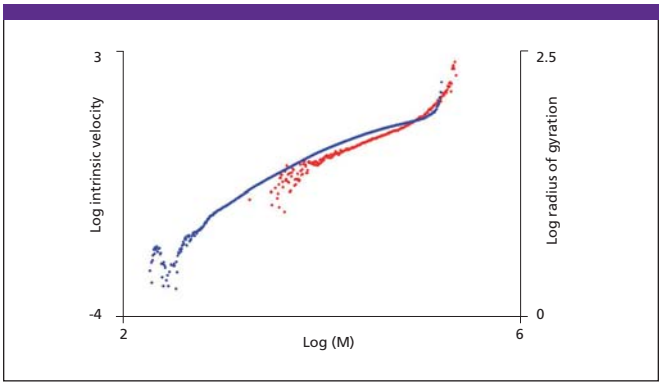

Figure 3: (Blue) Overlaid Mark-Houwink and (red) conformation plots for the pectin sample.

Polymer Laboratories, Inc.
Amherst Fields Research Park, 160 Old Farm Road
Amherst, MA 01002
e-mail plinfo@polymerlabs.com, www.polymerlabs.com