

Analysis of food additives by GPC/SEC

Application compendium

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The Measure of Confidence



Agilent Technologies

Contents	Page
Introduction	3
Pectins	4
Carboxymethyl cellulose.....	6
Starch.....	7
Corn flour.....	8
Pullulan and dextran.....	9
Gelatin.....	11
Gums.....	12
Other food solutions.....	13
Ordering information and further reading.....	15

Polymer Laboratories was formed in 1976 to offer high quality columns, standards, instruments, and software for GPC/SEC. For over 30 years the company developed many market-leading products, including PLgel, PL aquagel-OH, PlusPore, PLgel Olexis, PolarGel columns, and EasiVial standards. Built on advanced in-house manufacturing technology, PL's products have the highest reputation for quality and performance, backed up by world-class technical and applications support.

With the acquisition of PL, Agilent offers an even wider range of GPC/SEC solutions for all types of polymer characterization of synthetic and bio-molecular polymers, with options for conventional GPC all the way up to complex determinations using multi-column and multi-detection methods.

Introduction

In food processing, formulation involves mixing active ingredients with one or more additives to increase the efficiency of the manufacturing process and improve the desirability of the food to consumers. For example, enhancements can preserve flavor or improve taste and appearance.

Other additives are used to modify taste, acidity, appearance, mouth feel and prolong shelf life. Additives that assist in manufacturing include anti-caking agents and flow improvers.

It is very important for food manufacturers that their products are consistent in taste, appearance and quality, and all these parameters can be affected by additives. Because many food additives are polymeric in structure they are best analyzed by gel permeation chromatography or size exclusion chromatography, which provide a thorough understanding of the characteristics of polymer additives.

This compendium describes some of the uses of Agilent columns and systems for the gel permeation chromatography of common food additives.



Pectins

Pectin is composed of a variety of complex heteropolysaccharides found naturally in fruits, such as apples, plums, grapes and cranberries (Figure 1). 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 heterogeneous nature, pectins adopt complex structures in solution. Applications of pectin are related to the formulation 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.

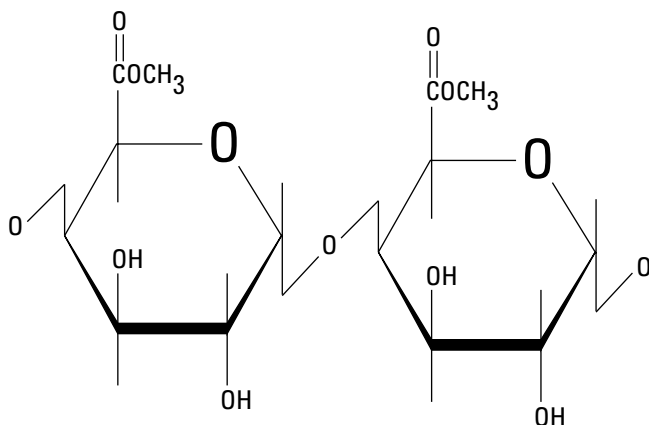


Figure 1. General structure of a pectin

A sample of pectin was analyzed on the Agilent PL-GPC 50 Integrated GPC/SEC System. The instrument was operated at 50 °C and incorporated a refractive index detector, an Agilent PL-GPC 50 Viscometer and an Agilent PL-GPC 50 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.

Figure 2 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 3 is the calculated molecular weight distribution.

Conditions (Figures 2 to 4)

Sample: Pectin at 2 mg/mL in the eluent
Calibrants: Agilent EasiVial PEO, 0.1 to 0.5 mg/mL
Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 7.5 x 300 mm (Part No. PL1149-6800)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ adjusted to pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Temp: 50 °C
Detection: PL-GPC 50, DRI, Viscometer, Dual Angle Light Scattering Detector

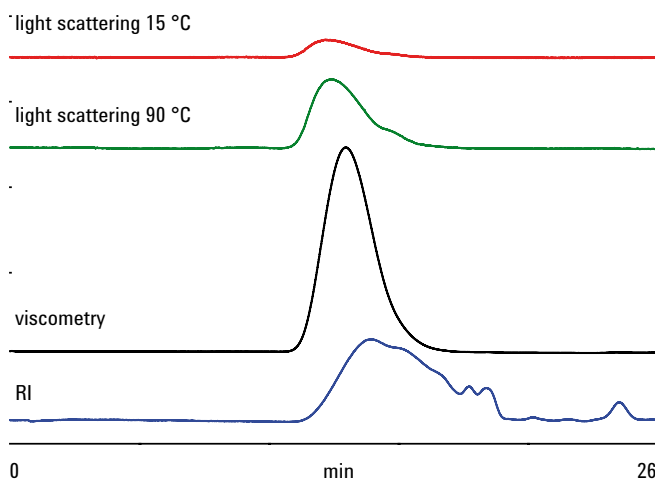


Figure 2. Triple-detector chromatograms of pectin (autoscaled) analyzed by the Agilent PL-GPC 50 with Agilent PL aquagel-OH MIXED-H two-column set

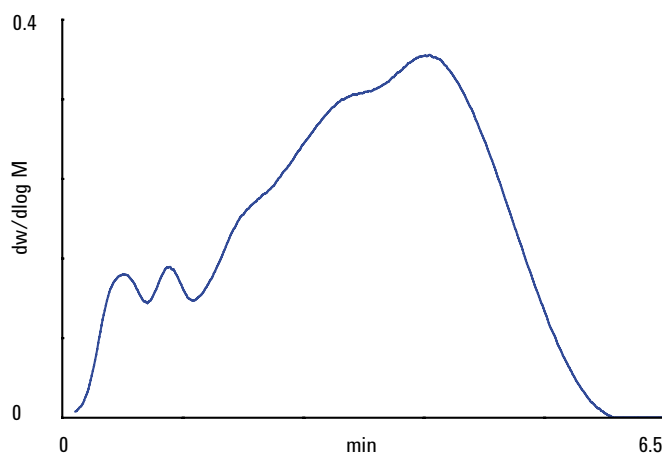


Figure 3. Molecular weight distribution plot calculated for the pectin

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

The PL-GPC 50 is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. When coupled with PL aquagel-OH MIXED-H 8 µm columns, PL-GPC 50 Viscometer and PL-GPC 50 Dual Angle Light Scattering Detector, the PL-GPC 50 makes maximum use of triple detection for the accurate determination of molecular weights of structurally complex and commercially important polymers. The wide resolving range of the columns allows complex natural materials such as pectin to be analyzed with confidence.

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 4.

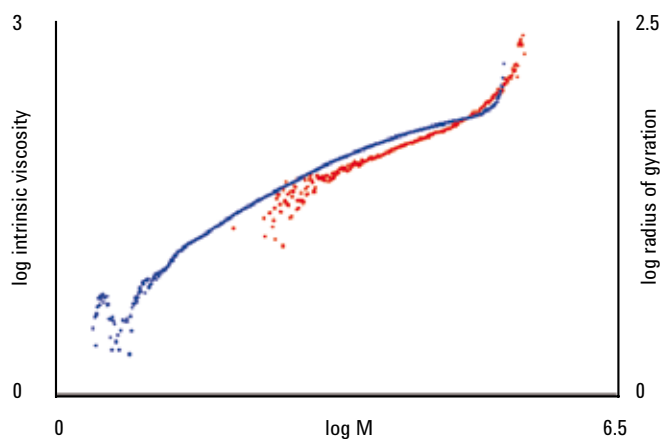
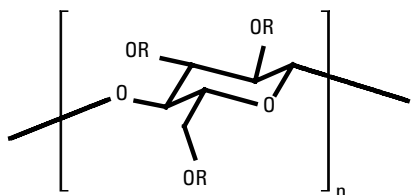


Figure 4. Overlaid Mark-Houwink and conformation plots for pectin

Carboxymethyl cellulose

Carboxymethyl cellulose (CMC) is a derivative of cellulose with carboxymethyl groups (CH_2COOH) attached at some of the hydroxyl groups that typically make up the cellulose backbone. The general structure is shown in Figure 5.



R = H or $\text{CH}_2\text{CO}_2\text{H}$

Figure 5. General structure of the CMC monomeric repeat unit

CMC has useful material properties such as high solution viscosity. This, coupled with the low toxicity and non-allergenic nature of the material, results in its widespread use within the food science arena.

Figure 6 shows the dual detector chromatogram for the CMC sample.

Figure 7 shows the molecular weight distribution calculated via the Universal Calibration, a technique utilizing the viscometer to determine molecular weights independent of the chemistry of the polymer calibrations employed.

Figure 8 shows the Mark-Houwink plot generated from the viscometry data. For samples of CMC, the segregation of carboxy and hydroxyl functionalities can influence the size of the molecules in solution. In this case, the curvature of the Mark-Houwink plot can provide information about the structural or chemical homogeneity as a function of molecular weight.

Conditions (Figures 6 to 8)

Sample:	Carboxymethyl cellulose
Sample Conc:	2.0 mg/L
Calibrants:	Agilent EasiVial PEG/PEO
Columns:	2 x PL aquagel-OH 30 8 μm , 7.5 x 300 mm (Part No. PL1120-6830)
Eluent:	0.2 M NaNO_3 , 0.01 M NaH_2PO_4 , pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	100 μL
Temp:	Ambient
Detection:	PL-GPC 50, DRI, Viscometer

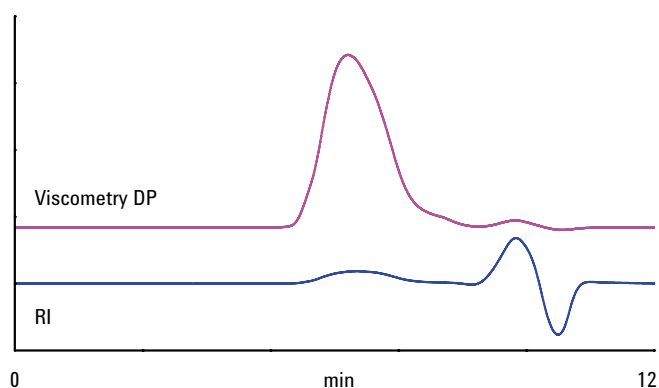


Figure 6. Refractive index/viscometry raw-data chromatograms obtained from a carboxymethyl cellulose sample analyzed on an Agilent PL-GPC 50 system with an Agilent PL aquagel-OH 30 column

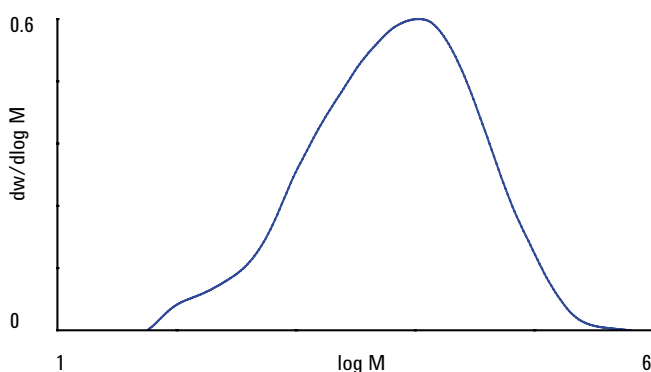


Figure 7. Molecular weight distributions obtained from carboxymethyl cellulose

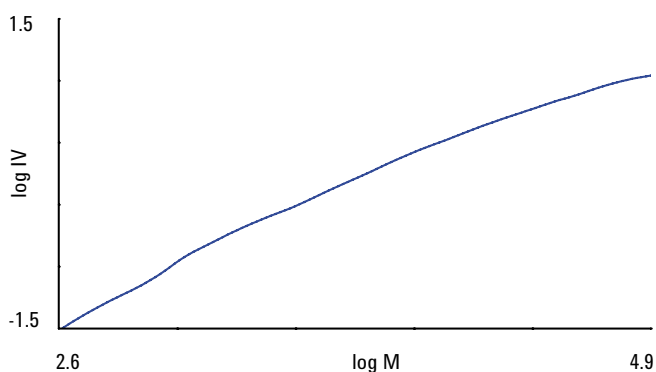


Figure 8. Mark-Houwink plot of a sample of carboxymethyl cellulose

Starch

Starches are polysaccharides that contain glucose polymers amylose and amylopectin in a ratio of about 30:70 depending on the source. They have a great many industrial applications, with a very important role in the food industry. The source of the starch leads to different properties and therefore different end uses in foods. For example, corn starch is suited for confectionary products whereas potato starches are used in processed meats. They are also used as thickening agents in cooking. The molecular distribution and weight of the polymer determines many of the final properties of the polymer and therefore the end-use suitability for different applications.

Gel permeation chromatography with the Universal Calibration, employing a viscometer in combination with a differential refractive index detector, is used to determine accurate molecular weights for biopolymers, such as starches, that are independent of the standards used in the column calibration. Two starches were analyzed using these techniques. Agilent PLgel Olexis columns were chosen for the investigation because they are designed for the analysis of very high molecular weight polymers such as starches. The column resolves up to 100,000,000 g/mol (polystyrene in THF), and is packed with 13 μm particles to optimize efficiency and resolution without the risk of sample shear degradation during analysis.

Figure 9 shows chromatograms of two different starches by refractive index and viscometry, Figure 10 their molecular weight distributions and Figure 11 the overlaid Mark-Houwink plots.

The two samples of starch analyzed by the Universal Calibration technique employing the Agilent 1260 Infinity Multi-Detector GPC/SEC System and PLgel Olexis columns showed stark differences in molecular weight distributions, with one of the samples having a bi-modal distribution. This accounted for the different thickening properties of the two materials. The variations in the Mark-Houwink plots indicated that the materials were structurally very different, presumably due to the fact the samples were obtained from two separate sources.

Conditions (Figures 9 to 11)

Sample: Starches at 2 mg/mL in the eluent
Columns: 3 x Agilent PLgel Olexis, 7.5 x 300 mm
(Part No. PL1110-6400)
Eluent: DMSO/DMAc (4:1) + 0.1% LiBr
Flow Rate: 1.0 mL/min
Temp: 60 °C
Detection: 1260 Infinity MDS incorporating viscometer and DRI

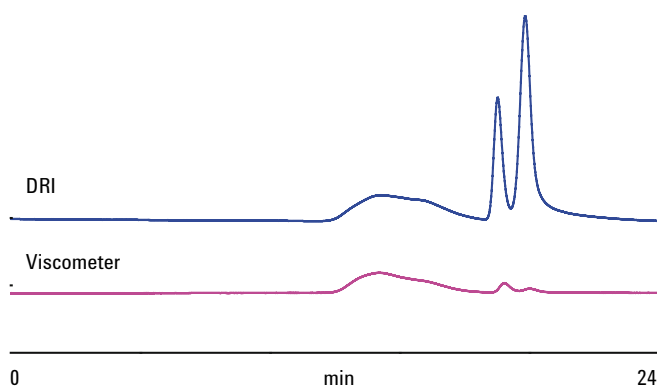


Figure 9. Viscometer and refractive index detection chromatograms for a starch on the 1260 Infinity MDS with an Agilent PLgel Olexis three-column set

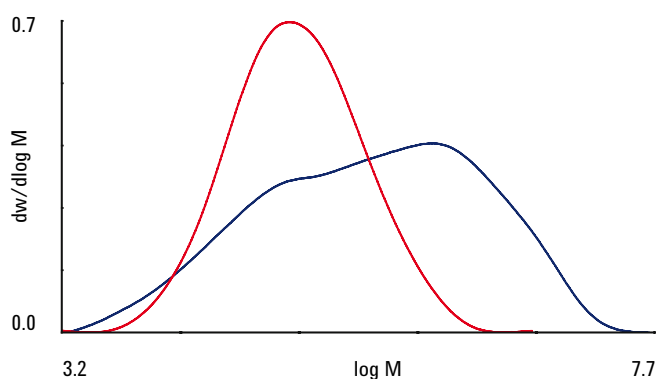


Figure 10. Overlaid molecular weight distributions (MWD) for two starch samples. Differences in MWD account for their different physical properties

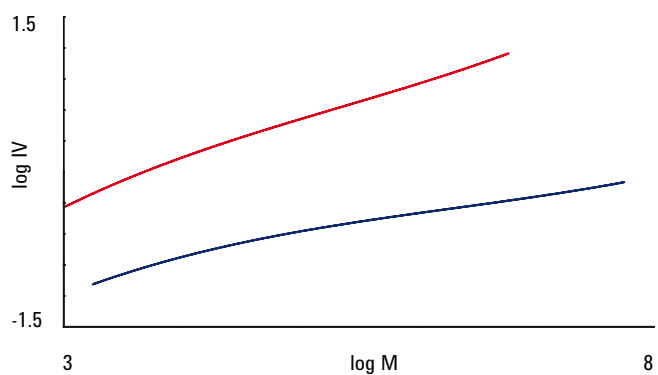


Figure 11. Overlaid Mark-Houwink plots for two starch samples reveals marked differences in their structure, with one of the samples having a much lower size in solution and therefore intrinsic viscosity than the other sample, which may be due to differences in the level of branching between the two materials under investigation

Corn flour

Corn flour or cornstarch is the starch of the maize grain, ground from the endosperm of the corn kernel. Starches contain two structurally different polysaccharides, amylose and amylopectin. In corn flour, the ratio of these materials is typically around 25:75. When mixed with water, corn flour behaves as a non-Newtonian fluid showing typical shear thickening behavior, giving way to gentle pressure but resisting sudden impact. Many uses of corn flour in the food industry rely on the thickening and anti-coagulant properties of the material.

Two samples of corn flour from different sources had displayed differing properties when used as thickening agents in a food application. It was thought that variations in levels of the linear amylose and the highly branched amylopectin polysaccharides were responsible for this behavior. To investigate the molecular structure of the materials, they were analyzed on an integrated GPC system. Molecular weight distributions were determined using the Universal Calibration method, and the structure of the samples was compared using the Mark-Houwink plot of \log (intrinsic viscosity) as a function of \log (molecular weight). Increased amount of branched material would result in a contraction in the molecular size of the materials with a downward deviation in the Mark-Houwink plot.

The corn flours were analyzed by an Agilent PL-GPC 50 fitted with an Agilent PL-BV 400RT viscometer and Agilent PLgel 10 μm MIXED-B columns, which provide high resolution of polymers with high molecular weights even in demanding eluents.

Figure 12 shows a chromatogram of a sample of corn flour. Clear differences in the molecular weight distributions of the samples are apparent in Figure 13, and the effect of changes to the content of amylose and amylopectin could be observed in the shifts of the Mark-Houwink plots (Figure 14).

The results show that the two samples of corn flour have markedly different sizes in solution, indicative of structural differences between the materials. This is most likely caused by variations in the ratio of amylose to amylopectin in the two samples. As a result of these size differences, conventional GPC employing only a refractive index detector would give an anomalous result for the two samples, as size is used to calculate the molecular weights calculated by conventional GPC.

Conditions (Figures 12 to 14)

Sample: Corn flour at 2 mg/mL in the eluent
Columns: 3 x PLgel 10 μm MIXED-B, 7.5 x 300 mm
(Part No. PL1110-6100)
Eluent: Dimethyl sulfoxide + 0.1% LiBr
Flow Rate: 1.0 mL/min
Temp: 50 °C
Detection: PL-GPC 50, DRI, Viscometer

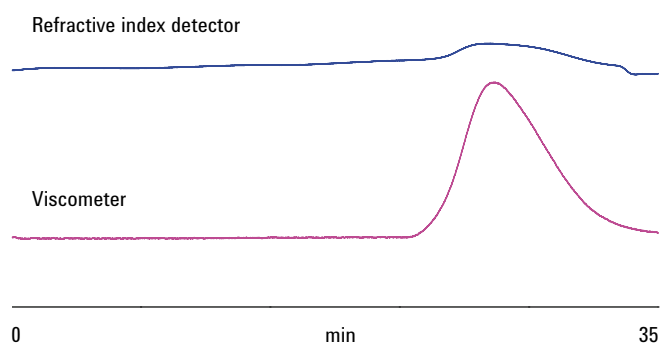


Figure 12. Example chromatograms of one of two corn flour samples produced by an Agilent PL-GPC 50 system with Agilent PLgel 10 μm MIXED-B columns

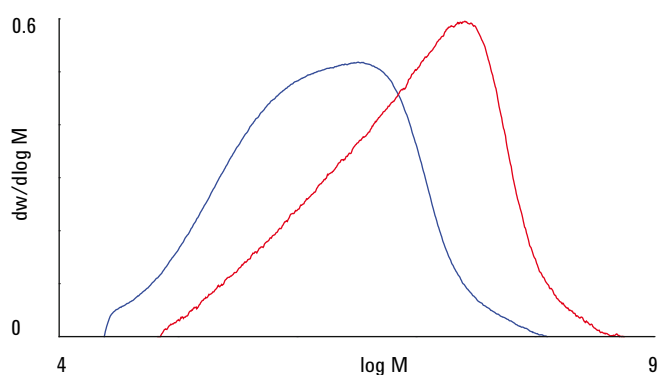


Figure 13. Overlaid molecular weight distributions for two corn flours

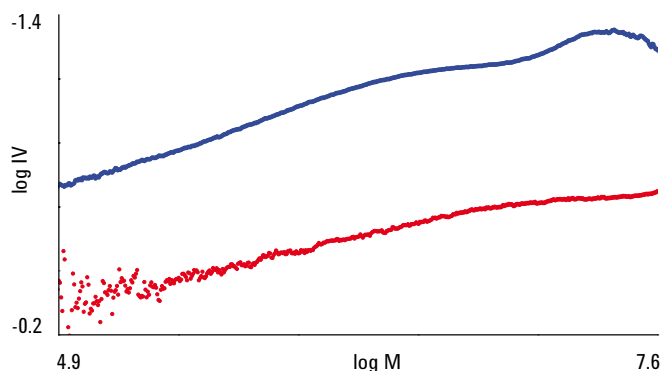


Figure 14. Overlaid Mark-Houwink plots for two samples of corn flour

Pullulan and dextran

Many polysaccharides 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 dextran, manufactured from sucrose by bacterial action, is a complex glucan with many differing components and 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.

Two samples of polysaccharide are analyzed by GPC viscometer; pullulan with a linear structure, and a highly branched dextran.

Figure 15 shows an example overlaid multi-detector chromatogram for a sample of pullulan polysaccharide. The material eluted as a broad peak, with a small late eluting peak on the DRI detector due to solvent imbalances.

Figure 16 is 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 17 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, with the data indicating slightly more branching at lower molecular weight.

Conditions (Figures 15 to 17)

Samples: Pullulan and dextran at 2 mg/mL in the eluent
Columns: 2 x Agilent PL aquagel-OH MIXED-M 8 μm , 7.5 x 300 mm (Part No. PL1149-6801)
Inj Vol: 200 μL
Eluent: 0.2 M NaNO_3 + 0.01 M Na_2HPO_4
Flow Rate: 1.0 mL/min
Temp: 40 $^\circ\text{C}$
Detection: 1260 Infinity MDS incorporating viscometer and DRI

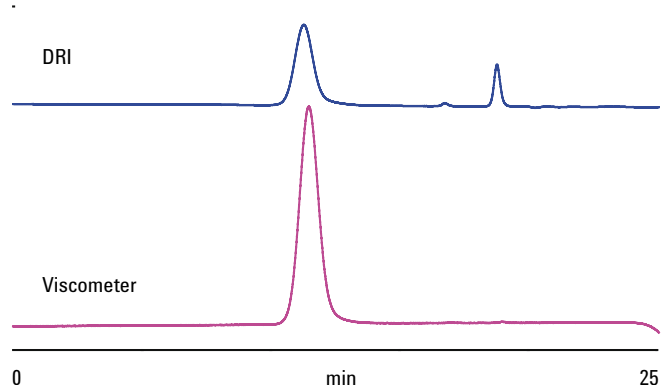


Figure 15. Overlaid multi-detector chromatogram for an example of pullulan polysaccharide on the Agilent 1260 Infinity MDS with Agilent PL aquagel-OH MIXED-M columns

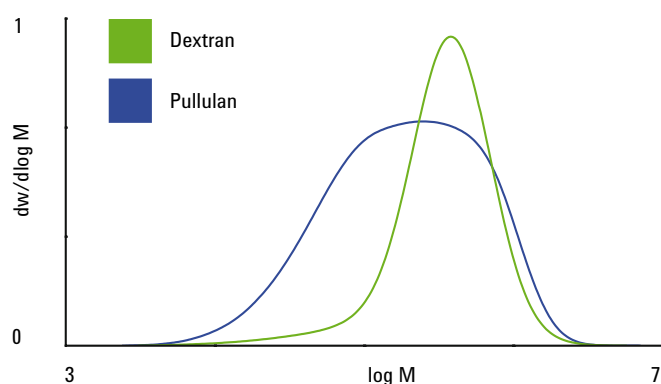


Figure 16. Overlaid multi-detector molecular weight distributions of two samples of polysaccharide

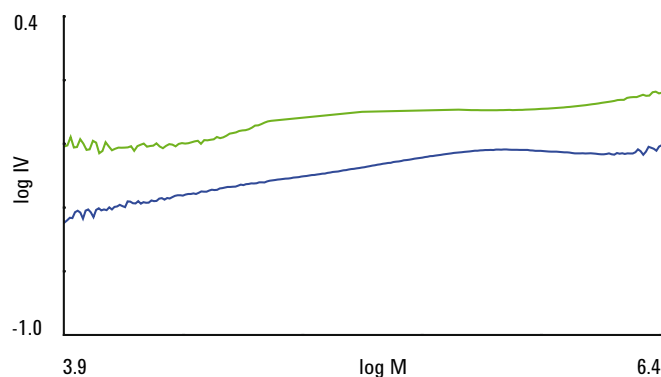


Figure 17. Overlaid Mark-Houwink plots for two polysaccharide samples

The data illustrate how multi-detector GPC employing the 1260 Infinity MDS can be used to clearly see structural differences between pullulan and dextran with a highly branched structure when coupled to a high resolution SEC column set capable of analyzing water-soluble polymers of quite high molecular weight.

Dextran can also be analyzed using single-detector GPC, as shown in Figure 18. In this analysis a dextran narrow polydispersity standard was used.

Conditions

Sample: Dextran narrow standard
Sample Conc: 2 mg/mL
Columns: 2 x PL aquagel-OH MIXED-M 8 μ m, 7.5 x 300 mm
(Part No. PL1149-6801)
Eluent: 0.2 M NaNO₃, 0.01 M NaH₂PO₄, pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Temp: 40 °C
Detection: DRI

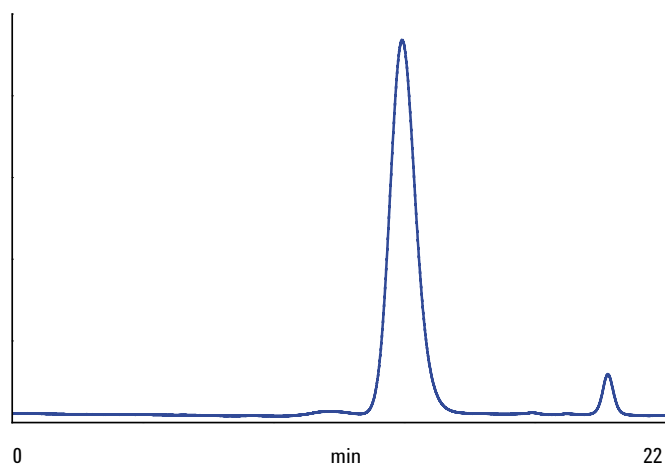


Figure 18. Typical chromatogram of a narrow polydispersity dextran on a two-column set of Agilent PL aquagel-OH MIXED-M columns

Gelatins

Food-grade gelatins are biologically derived materials used in the food industry as thickening agents. SEC analysis of gelatins yields critical molecular weight information upon which the physical properties of the polymer (such as the setting properties) depend. Linear Agilent PL aquagel-OH MIXED-H 8 μm columns were used. These columns resolve up to 10,000,000 g/mol (polyethylene glycol/oxide equivalent), offering high performance columns with excellent resolution over a very wide range of molecular weights, simplifying column selection and providing a versatile analytical system.

The eluent was prepared as a buffer with its pH adjusted by the addition of 0.1 M NaOH. The samples were accurately prepared as 1.0 mg/mL solutions in the eluent. The light scattering detector was first calibrated using a pullulan polysaccharide standard prepared at 1.0 mg/mL. From the known concentration, M_p and dn/dc of the calibrant, the detector constants and inter-detector volume for the system were calculated allowing molecular weight calculations to be performed.

From the RI chromatogram, dn/dc was calculated for the gelatin sample as the sample had been prepared at known concentration. This value of dn/dc was then used to calculate a bulk M_w value from the 90° and the 15° light scattering data.

The RI and light scattering data was also used to perform an SEC slice-by-slice molecular weight calculation for the gelatin sample using both LS signals. The bulk M_w values were 174,000 (90°), 189,850 (15°) and 184,800 (SEC).

Figure 19 shows the RI and the 90° and 15° light scattering data for the gelatin sample. Light scattering detection is more sensitive to higher molecular weight species, hence the 90° and 15° light scattering chromatograms placed more emphasis on high molecular weight material than the RI chromatogram. The RI chromatogram also contained a negative peak due to compositional differences between the sample, solvent and eluent, which was not observed by light scattering.

Conditions

Sample: Gelatin at 2 mg/mL in the eluent
Columns: 2 x PL aquagel-OH MIXED-H 8 μm , 7.5 x 300 mm (Part No. PL1149-6800)
Eluent: Water + 0.2 M NaNO_3 + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: 1260 Infinity MDS incorporating dual angle light scattering and DRI

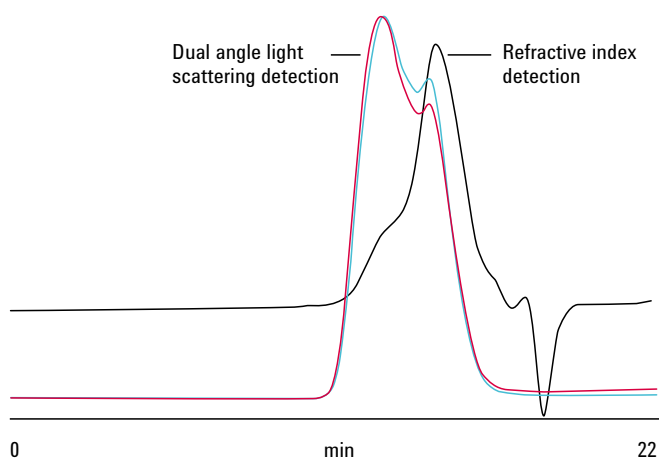


Figure 19. Refractive index, and 90° and 15° light scattering data for a gelatin sample on a 1260 Infinity MDS GPC/SEC System with an Agilent PL aquagel-OH MIXED-H two column set

The wide molecular weight operating range of PL aquagel-OH MIXED-H 8 μm columns makes them particularly suited to the analysis of water soluble polymers with intermediate to high molecular weight. The use of a simple buffer solution as the eluent for the analysis of gelatins reduces interaction between the sample and the columns ensuring that good chromatography is obtained.

Gums

Gums are complex polysaccharides used widely in the food industry as viscosity modifiers or gelling agents that provide characteristic shape and consistency to many foods. Most are derived from natural products, such as seaweed or locust bean. Others are extracted from microbial fermentation, or from animal tissue. Conversely, cellulose gum can be synthesized by reacting cellulose with chloroacetic acid. The physical properties and processibility of these water-soluble polymers are related to their molecular weight distribution, which can be determined by aqueous size exclusion chromatography.

Gum Arabic is a natural gum made from the hardened sap of acacia trees. It is produced across the African Sahel and the Middle East, and is an important ingredient in soft drinks and candies. The proportions of constituent polymers in gum Arabic vary widely. A comparison of the molecular weight distributions of 'good' and 'bad' samples shows clear differences between two batches of gum Arabic, the 'bad' sample having considerably greater high-molecular-weight material (Figure 20).

The difference between two batches of gum Arabic designated 'good' and 'bad' can be seen clearly by overlaying their molecular weight distributions (Figure 20).

Conditions

Sample: Gum Arabic at 2 mg/mL in the eluent
Columns: 2 x PL aquagel-OH 60 8 μm , 7.5 x 300 mm (Part No. PL1149-6860)
1 x PL aquagel-OH 40 8 μm , 7.5 x 300 mm (Part No. PL1149-6840)
Eluent: 0.2 M NaNO_3 , 0.01 M NaH_2PO_4 , pH 7
Flow Rate: 1.0 mL/min
Detection: DRI

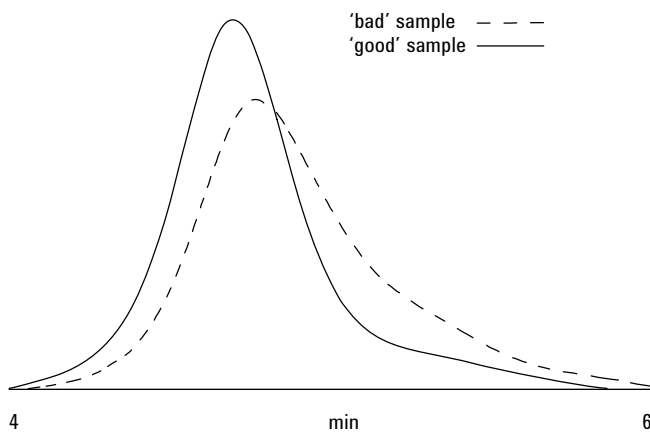


Figure 20. Overlay chromatogram showing molecular weight distribution of two batches of gum Arabic produced on an Agilent PL aquagel-OH three column set

Other Agilent food solutions

With the globalization of the food chain, protecting consumers and brands becomes more demanding. Today, the food and agriculture industry faces ever-increasing requirements for more sensitive and productive analytical solutions. You are committed to providing food, produce and beverages of consistent quality and uncompromising safety. We at Agilent Technologies are committed to provide the industry with products and services to help you deliver what your customers demand. Our instruments, systems, and supplies are used throughout the food production chain, including incoming inspection, new product development, quality control and assurance, and packaging. Here are a few examples.

Pesticides and residues

You can achieve a 10x productivity gain by replacing multiple 50 minute GC and GC-MS/SIM analyses with one 15 minute full scan GC/MS analysis for non-targeted pesticide screening. Just use an Agilent J&W HP-5ms column, deconvolution reporting software, a capillary flow three-way splitter, and trace on detection.



Trace metal contaminants

Using a simple procedure based on microwave digestion and single He-mode ICP-MS analysis, food samples can be quickly and accurately analyzed for trace and major element concentrations without the need for multiple sample preparations and analytical techniques. The Agilent 7700x using He mode alone can provide sensitive, accurate, interference-free analysis of a variety of metals in common foods.



Natural compounds and additives

For amino acid analysis, using HPLC with ZORBAX Rapid Resolution High Definition Eclipse Plus C18 columns with 1.8 μm particles enables analysis cycle time to be more than cut in half, from about 35 minutes to about 13.5 minutes. In addition, peak shapes of early eluting aspartic acid and glutamic acid are improved, as is the reproducibility of the secondary AAs, and that of the slowest AA to react, lysine. Linearity is excellent (nearly 1) and average peak area reproducibility is below 2%.



Bioanalysis

Accurate identification of wheat varieties is of paramount importance to the milling industry. The Agilent 2100 bioanalyzer and the Protein 230 analyze wheat proteins for varietal identification. The ease-of-use and total analysis time of less than 50 minutes makes it most suitable for mill in-take use, enabling millers to make more confident decisions in accepting grain consignments.

For a comprehensive summary of Agilent food solutions get your copy of the Agilent Food Compendium (publication number 5988-4450EN) at agilent.com

Ordering information

The following products are featured in this application compendium. For a full list of GPC/SEC part numbers, visit

agilent.com/chem/store

Columns	
Description	Part No.
Agilent PL aquagel-OH 30 8 µm, 7.5 x 300 mm	PL1120-6830
Agilent PL aquagel-OH 40 8 µm, 7.5 x 300 mm	PL1149-6840
Agilent PL aquagel-OH 60 8 µm, 7.5 x 300 mm	PL1149-6860
Agilent PL aquagel-OH MIXED-H 8 µm, 7.5 x 300 mm	PL1149-6800
Agilent PL aquagel-OH MIXED-M 8 µm, 7.5 x 300 mm	PL1149-6801
Agilent PLgel 10 µm MIXED-B, 7.5 x 300 mm	PL1110-6100
Agilent PLgel Olexis, 7.5 x 300 mm	PL1110-6400

Standards	
Description	Part No.
Agilent EasiVial PEO	PL2070-0200
Agilent EasiVial PEG/PEO	PL2080-0200

Instruments	
Description	Part No.
Agilent PL-GPC 50 Integrated GPC/SEC System	G7810A
Agilent PL-GPC 50 Dual Angle Light Scattering Detector	G7812A
Agilent 1260 Infinity Multi Detector Suite	Contact your local sales office or distributor for different platform options

Suggestions for further reading

Agilent has published application compendia on biodegradable polymers, engineering polymers, polyolefin analysis, and low molecular weight resins. In addition, we also offer a comprehensive and informative range of literature for all aspects of GPC/SEC, including application notes, datasheets and technical overviews.

Publication	Publication number
Introduction to GPC/SEC	5990-6969EN
GPC/SEC column selection guide	5990-6868EN
Food application compendia	5991-2029EN
Energy and chemicals application compendia	5991-2517EN
Pharmaceuticals application compendia	5991-2519EN
Biodegradable polymers	5990-6920EN
Engineering polymers	5990-6970EN
Elastomers	5990-6866EN
Polyolefin analysis	5990-6971EN
Excipient analysis	5990-7771EN
Low molecular weight resins	5990-6845EN
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GPC wallchart	5990-6882EN

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