Biodegradable polymers - analysis of biodegradable polymers by GPC/SEC

Application compendium

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

The Measure of Confidence

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Contents Page

Studying biodegradable polymers

Biodegradation is the degradation of a material by environmental factors such as sunlight, temperature changes or the action of microbes. In polymer science and engineering, the design of polymers susceptible to biodegradation is of increasing importance for two reasons – polymers that degrade naturally in the body to harmless products may be used in biological devices and in drug delivery, and polymers that break down in the environment are significantly 'greener' than traditional plastics.

Biodegradation is key to the suitability of materials for use in drug delivery devices or in temporary structures within the body, such as sutures. For these applications, the ability of the body to naturally break down the material used either as part of the application or post-event is very important, making the removal of the polymer simply a case of allowing the natural process of degradation to occur. Many materials are being investigated for these applications as medical science progresses.

The landfill crisis has made the production of non-polluting polymers for packaging and engineering uses a high priority. These materials need to be able to perform their function, but also break down in the environment with time, a difficult proposition.

For these materials, the rate of degradation and therefore the lifetime and performance of the polymer in the natural environment is related to the length of the polymer chains in the material, with degradation leading to scission of the polymer chains and a shortening of their length.

Gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC), a well-known technique for determining the molecular weight distribution of polymers, is therefore key to studying biodegradable materials by giving an insight into the rate at which a material might degrade, and revealing the presence of degraded polymer chains in a sample.

This application compendium shows examples of GPC applications involving different biodegradable polymers, derived from synthetic and natural sources.

Agilent Technologies produces the most extensive range of GPC/SEC columns, standards and instruments that are ideally suited to the analysis of biodegradable polymers.

Agilent's columns are the most stable available, and include ranges suited for use in organic and aqueous eluents, solvent mixtures and high polarity organic eluents, covering the requirements of the diversity of biodegradable materials. With extensive options in particle and pore size, Agilent's columns can be specifically selected to match the molecular weight of the material under investigation, thereby ensuring that the best quality data is obtained from the GPC/SEC experiment.

Agilent's GPC/SEC columns are the most rugged and reliable on the market, making them ideal for applications that rely on extremely reproducible analysis such as in quality control environments.

Given that many biodegrabale materials are destined for use in vivo, ensuring the quality of materials is of the upmost importance.

Agilent also manufactures narrow polydispersity standards with very highly characterized molecular weights that are used as calibration standards in the GPC/SEC analysis of biodegradable polymers.

Complementing Agilent's column technology is the most extensive collection of integrated GPC/SEC instrumentation on the market covering the temperature range from ambient to 220 °C.

These instruments allow all forms of the GPC/SEC experiment to be performed and can be used to analyze the complete range of biodegradable materials. Multiple detection options can be included in the instruments, such as light scattering and viscometry, and dedicated analysis software is available that allows the biodegradation properties of the materials to be monitored.

Agilent's complete range of columns and instrumentation offer a clear advantage in the analysis of biodegradable polymers.

Narrow dispersity polymer calibrants

Synthetic polymers

Poly(lactide-*co*-glycolide)

Application area: Drug delivery

Poly(lactide-*co*-glycolide) copolymers have found extensive applications in the pharmaceutical industry. The molecular weight distribution of the polymer can affect the properties of the end product, and is therefore of interest in both the areas of development and quality control.

The copolymer is quite polar in nature, but can be dissolved in several solvents suitable for gel permeation chromatography (GPC), notably tetrahydrofuran (THF) and chloroform.

Low boiling solvents like chloroform can suffer from outgassing effects. When employing refractive index detection, this can lead to chromatograms with noisy or drifting baselines. The Agilent 380-ELSD and Agilent 385-ELSD (cooled) evaporative light scattering detectors, on the other hand, always deliver baselines which are stable and drift-free. Furthermore, due to its evaporative nature, it provides chromatograms which are free from system peaks around total permeation which are commonly associated with refractive index detectors. Agilent's 380-ELSD and 385-ELSD also offer superior sensitivity compared to refractive index.

Poly(lactide-*co*-glycolide) copolymers are relatively low in molecular weight.

The Agilent PLgel 5 µm MIXED-D columns with their high efficiency (>50,000 plates/meter) and broad resolving molecular weight range (up to 400,000 daltons relative to polystyrene), are the columns of choice for this application.

Figure 1 shows a typical raw data chromatogram for a poly(lactide-co-glycolide) sample.

Columns: 2 x PLgel 5 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent: Chloroform
Flow Rate: 1.0 mL/min
Detector: Agilent ELSD

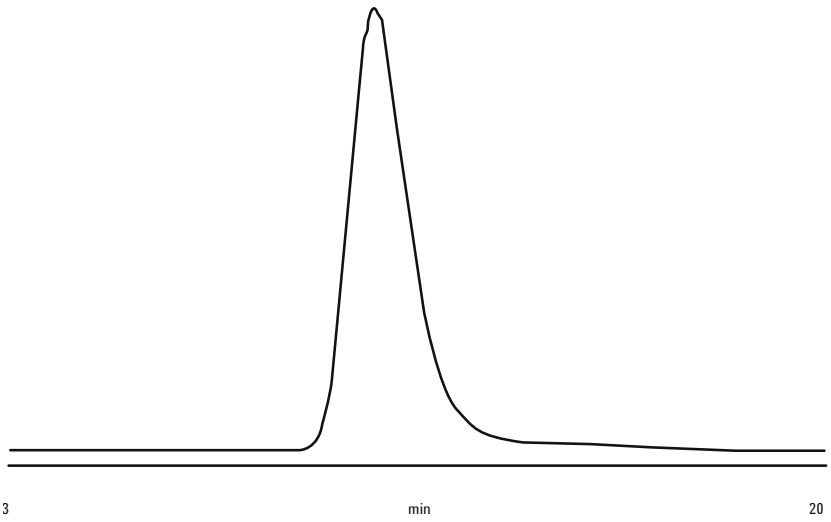


Figure 1. *Typical raw data chromatogram for a poly(lactide-co-glycolide) sample showing a typical gaussian peak shape*

Polycaprolactam

Application area: Drug delivery

Traditional drug delivery systems, such as the oral contraceptive pill, have a major disadvantage - the release of the active species is very non-linear, with typically a high dosage at the time of introduction followed by a steady decline as the drug is metabolized. A release profile of this kind is inefficient. Ideally, the dosage of the active compound into the body should remain at a constant level during treatment. The controlled delivery of drugs in vitro to produce linear dosing regimes is a major goal of therapeutic research. Polycaprolactam is a well-known polymer that biodegrades by enzymatic cleavage of ester bonds under conditions found within the human body. Introducing an active drug contained in a matrix of polycaprolactam into the body leads to the steady release of drug as the polymer matrix degrades. Appropriate inclusion of the drug into the matrix controls the rate of release

A critical parameter controlling the rate of degradation of biodegradable polymers is the molecular weight of the starting material. The higher the average molecular weight, the slower the rate of biological degradation. Measuring the molecular weight distributions of biodegradable polymers by gel permeation chromatography (GPC) is a critical part of research into controlled drug release with polymers. The chromatogram below shows polycaprolactam obtained in THF using two Agilent PLgel 5 µm MIXED-C columns. The polymer eluted as a broad peak with an average molecular weight of 80,000 g/mol and a polydispersity of 2.5.

Sample:	Polycaprolactam
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm (Part No. PL1110-6500)
Eluent:	THF (stabilized)
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Detector:	RI

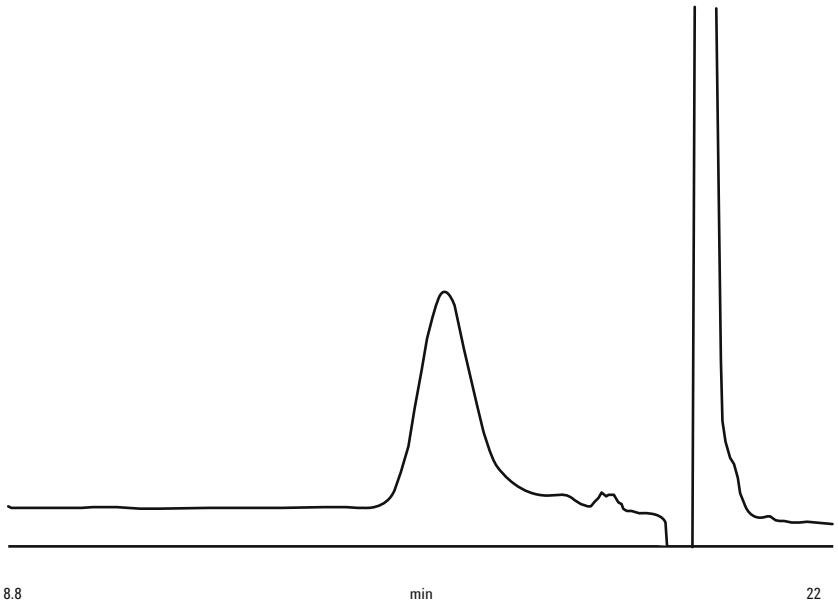


Figure 2. *Typical raw data chromatogram for a sample of polycaprolactam containing low molecular weight components and showing a large system peak*

Polyvinyl alcohol

Application areas: Adhesive, surfactant, surface properties

Fully or partially hydrolyzed grades of polyvinyl alcohol are normally specified according to their viscosity in solution. Aqueous SEC can be used to characterize these polymers in terms of molecular weight distribution. Three samples with the same degree of hydrolysis were compared by overlaying their molecular weight distributions. This is a convenient method of fingerprinting materials for quality control, and is more informative in production control and end-use performance evaluation than single point viscosity measurements.

Calibrants:	Pullulan polysaccharides
Columns:	2 x Agilent PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent:	0.25M $NaNO_3$, 0.01M NaH_2PO_4, pH 7
Flow Rate:	1.0 mL/min
Detector:	RI

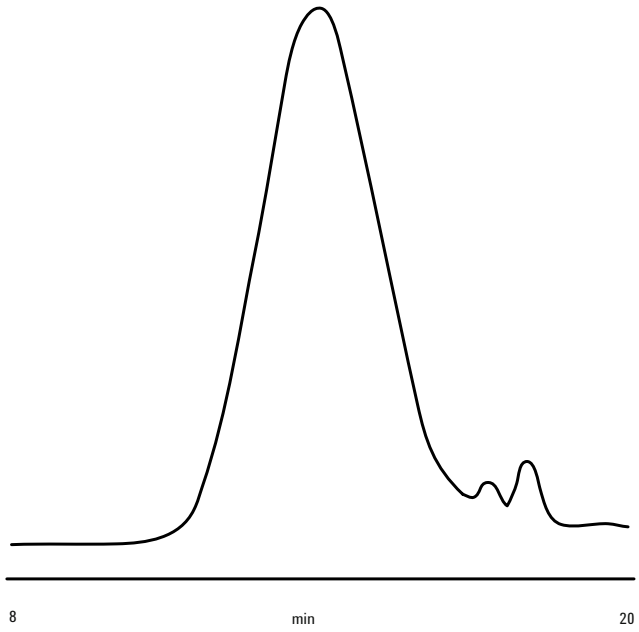


Figure 3. *Raw data chromatogram (sample A) showing the presence of low molecular weight components along with the polymer peak*

Table 1. Correlation of GPC results with polymer specification for PVA

Sample	Viscosity (mPa.s)	Mn	Mw
A	4.0	9771	29470
B	10.0	23339	80174
C	20.0	31210	102309

Polyethylene glycol (PEG)

Application areas: Excipient, dispersant, antifreeze

Polyethylene glycols are inert, water-soluble and biodegradable polymers used in a range of applications from medical formulations, protein conjugates to cosmetic products and antifreeze solutions. Manufactured by living polymerization processes, PEGs have narrow molecular weight distributions with physical properties controlled by their molecular weight.

Agilent PL aquagel-OH 30 8 µm high performance columns are ideal for relatively low molecular weight separations, combining low exclusion limit, high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution.

The separation below shows a range of PEG samples.

Columns: 2 x PL aquagel-OH 30 8 µm, 300 x 7.5 mm (Part No. PL1120-6830)
Eluent: Water
Flow Rate: 1.0 mL/min
Detector: RI

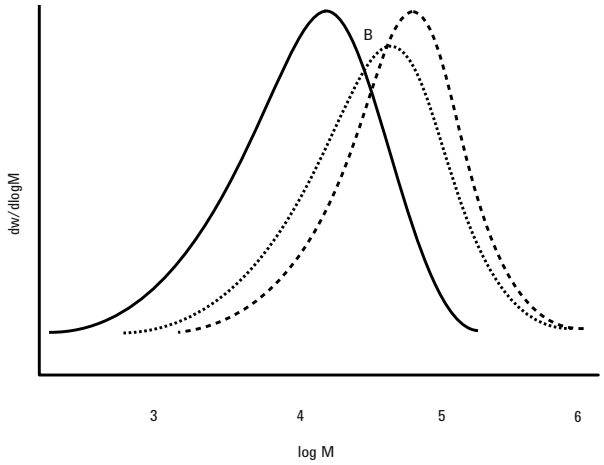


Figure 4. *Example molecular weight distributions of the three polyvinyl alcohols with very different physical properties*

Columns: 2 x PL aquagel-OH 30 8 µm, 300 x 7.5 mm (Part No. PL1120-6830)
Eluent: Water
Flow Rate: 1.0 mL/min
Detector: RI

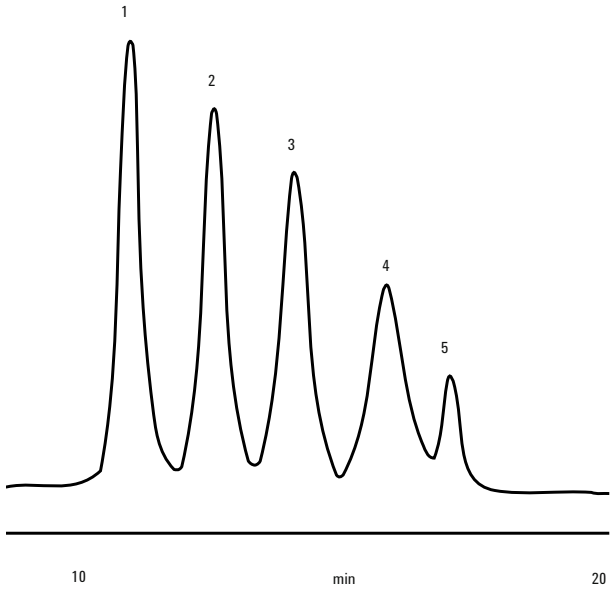


Figure 5. *Resolution achieved in the separation of five PEG samples used for calibration*

Naturally-occurring polymers

Natural rubber

Application area: Engineering material
A biodegradable polymer produced from tree sap, natural rubber is an elastomer that has many uses in a wide range of industrial and household products. It is degraded slowly by bacterial action.

Solutions of natural rubber samples are generally very difficult to prepare for GPC due to the fact that the polymer contains relatively high levels of 'gel', which is partially crosslinked. An aliquot of the eluent is added to the weighed sample. It is allowed to swell and dissolve overnight, and then the gel material is filtered out (0.5 µm) prior to GPC analysis.

In this case, the actual polymer concentration can be significantly lower than the original concentration prepared depending on the gel content of the sample, and therefore detector response, usually RI, tends to be quite poor. ELSD exhibits significantly increased sensitivity compared to an RI and subsequently gives much greater response for this application. In addition, RI baseline drift, which commonly occurs, is very much emphasized when the actual peak response is so small.

ELSD always gives a flat baseline which, together with the improved response, makes baseline and peak setting much more reliable for GPC calculations.

A further problem with RI is sensitivity to system peaks around total permeation, which usually occurs even when samples are prepared in an aliquot of the eluent. These system peaks can interfere with low molecular weight components which are commonly found in natural rubber samples. This situation is very much improved when ELSD is employed, as system peaks are eliminated due to evaporation, leaving unadulterated sample peaks in the additives region.

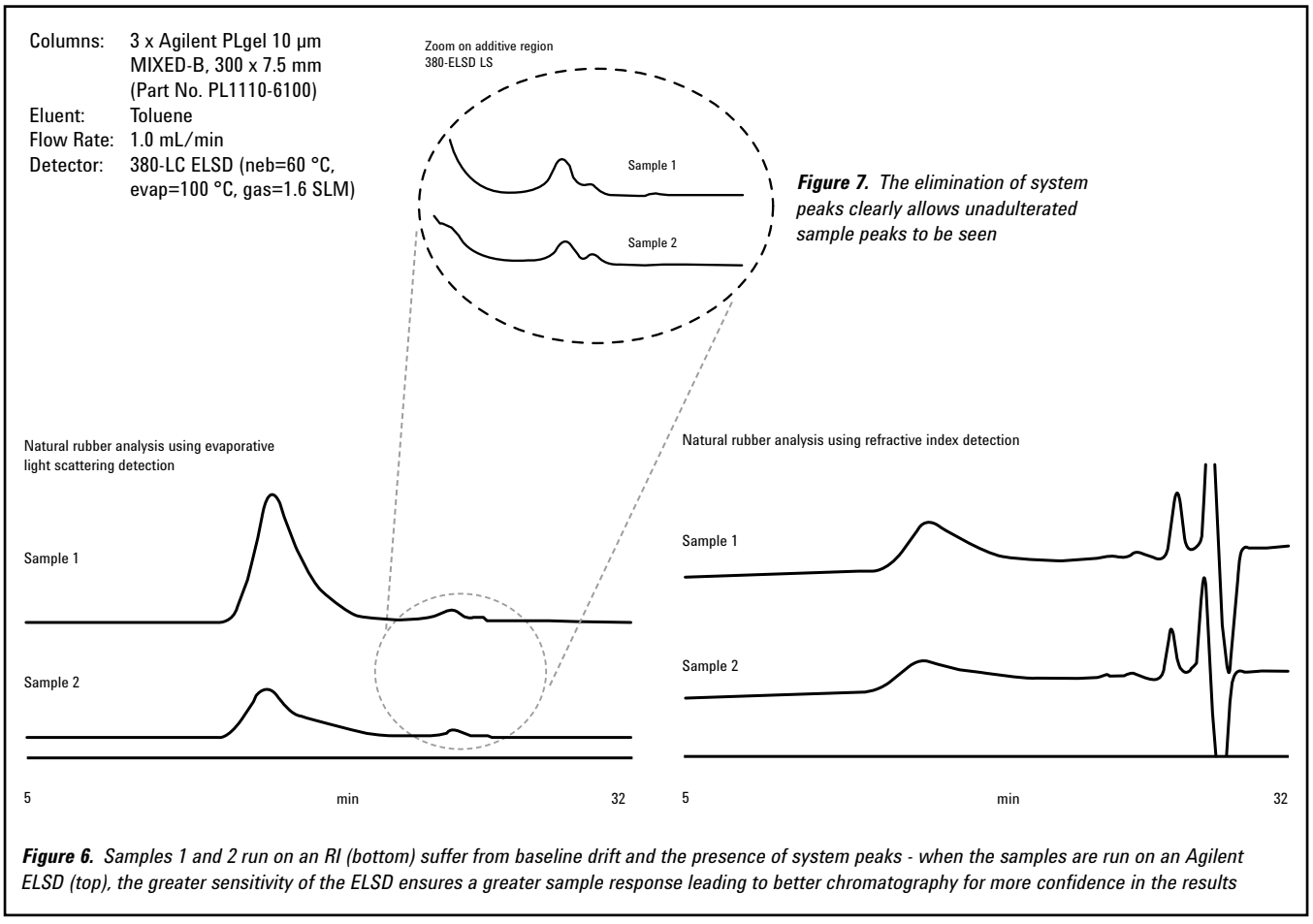


Figure 7. *The elimination of system peaks clearly allows unadulterated sample peaks to be seen*

Figure 6. *Samples 1 and 2 run on an RI (bottom) suffer from baseline drift and the presence of system peaks - when the samples are run on an Agilent ELSD (top), the greater sensitivity of the ELSD ensures a greater sample response leading to better chromatography for more confidence in the results*

Polyacrylic acid

Application areas: Adhesive, water treatment

Polyacrylic acid is a biodegradable water soluble polymer with numerous industrial applications, including as a super adsorbent (e.g. in disposable nappies), in water treatment as a metal ion scavenger and in the treatment of metal surfaces prior to coating.

The molecular weight distribution (MWD) of this material is an important parameter, as it strongly affects the end use properties of the polymer. Aqueous SEC is an ideal analytical tool for the measurement of the MWD of polyacrylic acid. Since polyacrylic acid is a polyelectrolyte, care must be taken in selecting the appropriate SEC conditions. In the SEC described below, a buffered mobile phase with a high electrolyte content was used to minimize non-size exclusion effects.

Agilent PL aquagel-OH MIXED-H columns were selected to provide good resolution over a wide molecular weight range. Column calibration was achieved using Agilent polyethylene oxide (PEO) EasiVial standards, see Figure 8.

Columns:	2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Sample Conc:	PEO standards: 0.1-0.5 mg/mL Polyacrylic acid: approx 0.2% w/v
Detector:	RI

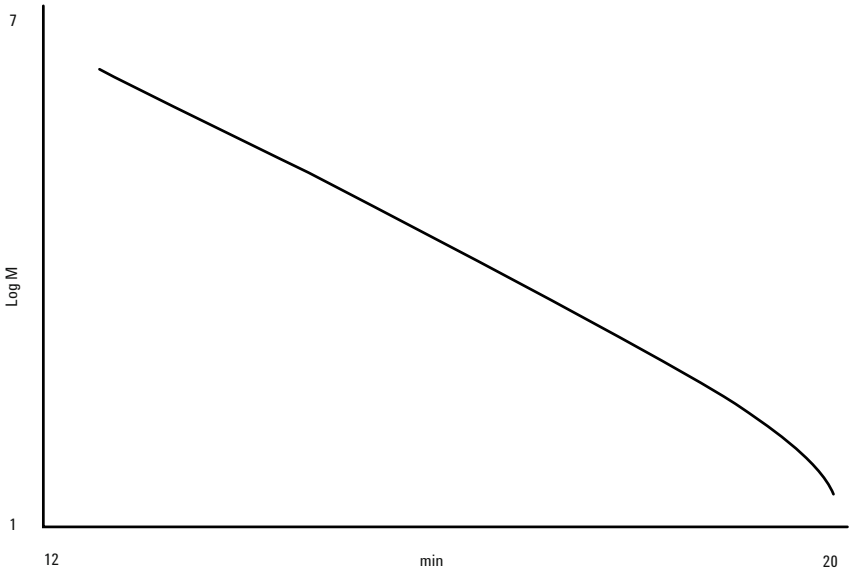


Figure 8. *PL aquagel-OH MIXED-H 8 µm column calibration using PEO EasiVial standards showing the relationship between retention time and the log of molecular weight*

EasiVial standards

EasiVial standards provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). Each vial contains a mixture of four individual, highly characterized, narrow dispersity standards. The amount of each individual standard is carefully controlled during manufacture, allowing their use in SEC-viscometry which requires accurate concentrations.

Refractive index chromatograms obtained from each PEO EasiVial are presented in Figure 9.

Three polyacrylic acid samples (A, B and C) were chromatographed and their corresponding molecular weight distribution compared, see Figure 10.

Table 2. Comparison of molecular weight averages for the three samples of polyacrylic acid

Sample	Mn (g/mol)	Mw (g/mol)	PD
A	33,450	89,430	2.67
B	7,990	14,930	1.87
C	7,880	13,490	1.71

Sample A was found to possess a significantly higher molecular weight distribution compared to Samples B and C, which were found to be similar. Consequently, Sample A was expected to possess significantly different rheological properties compared to the remaining two samples. Closer examination of the samples showed Sample A to be significantly more viscous than B and C, which were similar. In addition, the MWD of Sample A was found to be bi-modal, which suggests that the sample may be a blend of more than one component. In conclusion, differences in the molecular weight distributions of the polyacrylic acids were identified. These differences were corroborated through visual examination of the samples' bulk viscosity.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Sample Conc: PEO standards: 0.1-0.5 mg/mL
Polyacrylic acid: approx 0.2% w/v
Detector: RI

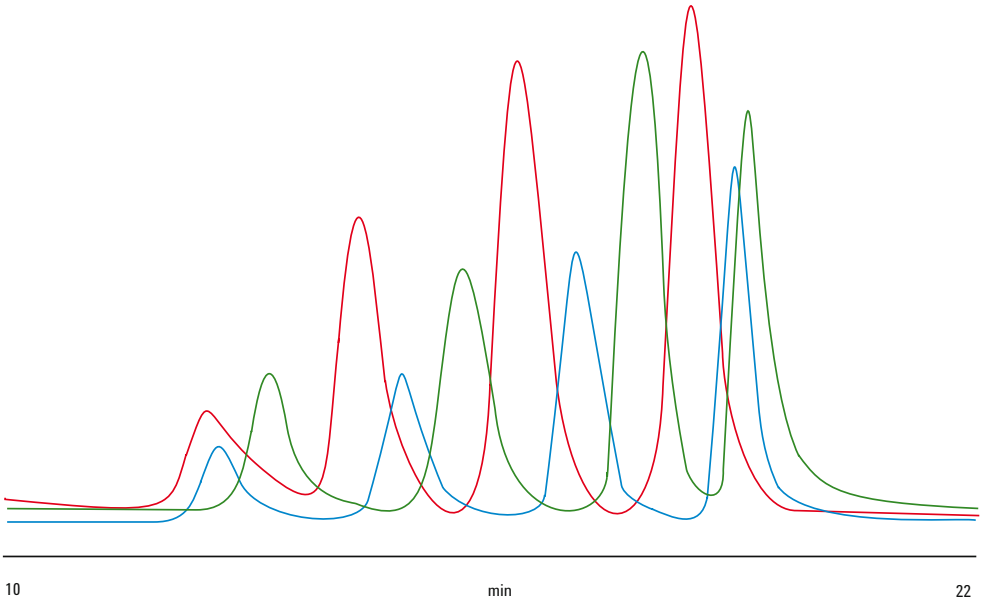


Figure 9. *Example chromatograms of PEO EasiVial standards used to create the calibration*

Columns:	2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Sample Conc:	PEO standards: 0.1-0.5 mg/mL
	Polyacrylic acid: approx 0.2% w/v
Detector:	RI

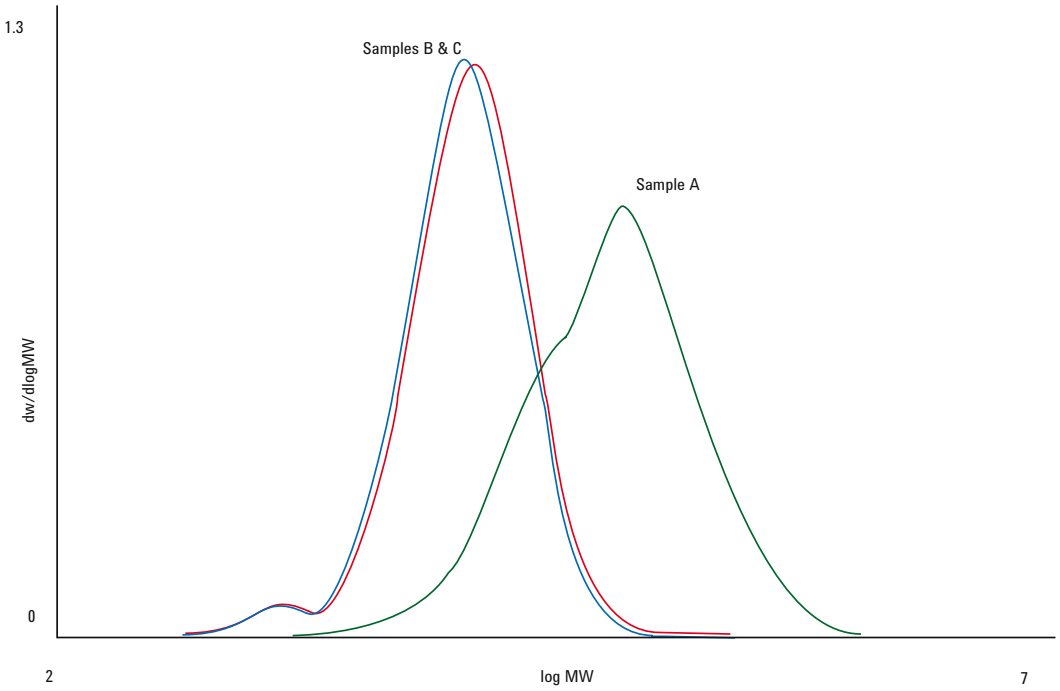


Figure 10. *Molecular weight distribution of three polyacrylic acid samples showing a clear difference between one of the samples (A) and the other two (B and C)*

Chitosan

Application areas: Drug delivery, paper production

Chitosan is a naturally-occurring polysaccharide made by alkaline N-deacetylation of chitin which is believed to be the second most abundant biomaterial after cellulose. The term chitosan does not refer to a uniquely-defined compound, but merely refers to a family of copolymers with various fractions of acetylated units containing both chitin and chitosan monomers.

The main interest in chitosan derives from its cationic nature in acidic solutions which provides unique properties relative to other polysaccharides, which are usually neutral or negatively charged. Application areas of chitosan include biomedical (e.g. wound healing, burn treatment and use as a hemostatic agent), paper production, textile finishes, photographic products, cements, heavy metal chelating agents and waste removal.

GPC/SEC can be used as a quality control tool for the determination of MW and MWD. Different molecular weights would be appropriate to particular applications.

Three grades of chitosan were analyzed using a column set comprising 2 x PL aquagel-OH MIXED 8 µm columns. These columns offer resolution over a wide molecular weight range (up to 10,000,000 relative to PEO/PEG).

Due to the cationic nature of the samples, they were prepared in strong acid and were allowed to stand overnight to aid dissolution. They were analyzed in 0.5M sodium nitrate buffer and at low pH.

Raw data chromatograms and weight average molecular weight values (Mw) for the three chitosan samples are shown below.

The system was calibrated with narrow pullulan polysaccharide standards and the resulting calibration curve is illustrated below.

Columns:	2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.5M $NaNO_3$, 0.01M NaH_2PO_4, pH 2
Flow Rate:	1.0 mL/min
Detector:	RI

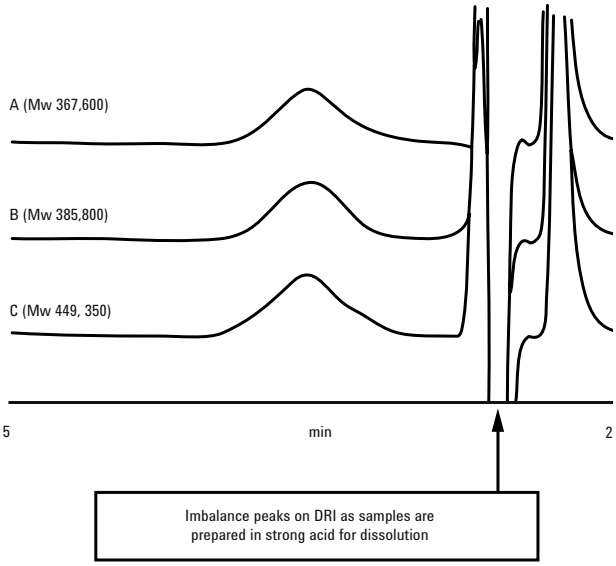


Figure 11. *Raw data chromatograms of three chitosan samples showing typical peak shapes with strong imbalance peaks due to dissolution conditions*

Columns:	2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.5M $NaNO_3$, 0.01M NaH_2PO_4, pH 2
Flow Rate:	1.0 mL/min
Detector:	RI

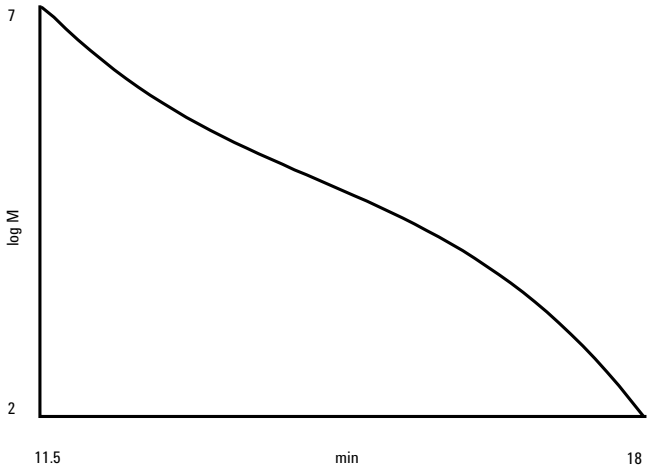


Figure 12. *SEC calibration showing the resolving range of the PL aquagel-OH MIXED-H 8 µm column set*

Cellulosic polymer

Application areas: Thickening agent and viscosity modifier
The most abundant component of plant matter, cellulose, can be modified to produce a number of biodegradable materials with useful properties.

Carboxymethyl cellulose (CMC) is a cellulose derivative with some of the hydroxyl groups of the glucopyranose monomers of cellulose modified to contain carboxymethl groups. CMC is a thickener used in the food industry where it has E number E466, and is also used to stabilize emulsions in ice cream. It is also a constituent of many non-food products, including toothpaste and water-based paints. Hydroxyethyl cellulose has some of the hydroxyl groups modified with ethyl chains, and is used as a gelling and thickening agent in cosmetics, cleaning solutions, and other household products.

Carboxymethyl cellulose

Calibrants:	Pullulan polysaccharides
Columns:	2 x Agilent PL aquagel-OH 60 8 µm, 300 x 7.5 mm (Part No. PL1149-6860)
	1 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent:	0.5M Na_2SO_4
Flow Rate:	1.0 mL/min
Detector:	RI

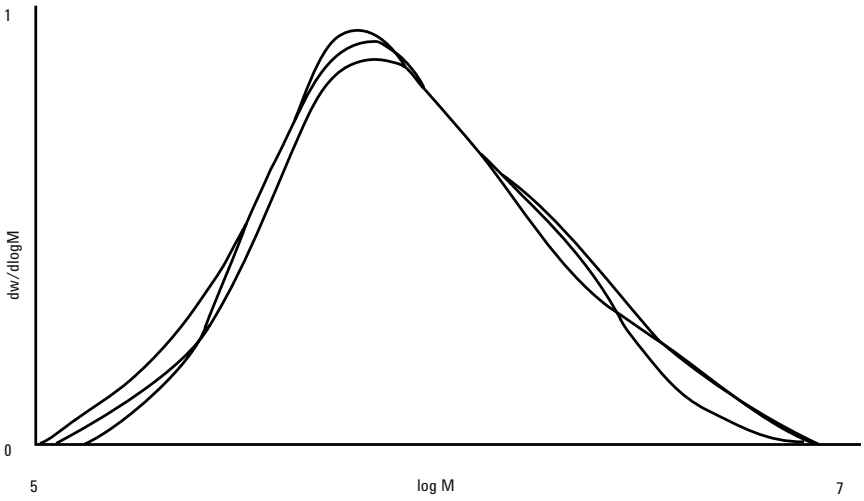


Figure 13. *Example molecular weight distributions of three samples of carboxymethyl cellulose with subtle differences between samples*

Hydroxyethyl cellulose

The correlation between the two measurements is good, showing that GPC is a viable measurement technique to viscosity when ensuring the quality of these samples.

Table 3. Correlation of viscosity data with GPC results for hydroxyethyl cellulose

	A	B	C
Viscosity Range (cps)	75-112	250-324	1500-2500
Mn	60,300	413,000	914,000
Mw	179,000	849,000	2,016,000
Mz	39,000	1,552,000	3,422,000

Calibrants:	Pullulan polysaccharides
Columns:	2 x PL aquagel-OH 60 8 µm, 300 x 7.5 mm (Part No. PL1149-6860)
	1 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent:	0.05M NaH_2PO_4, 0.25M NaCl, pH 7
Flow Rate:	1.0 mL/min
Detector:	RI

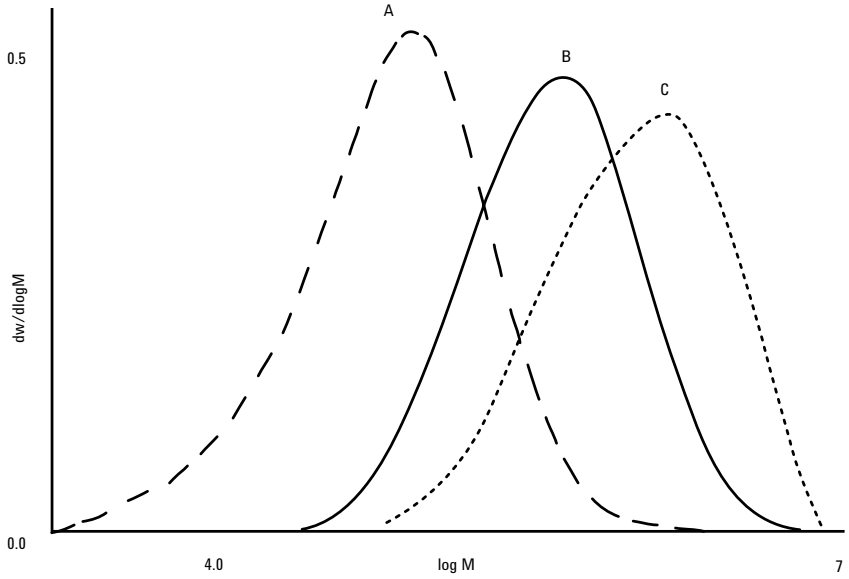


Figure 14. *Example molecular weight distributions of three samples, A, B and C with very different properties*

Methyl cellulose

Application areas: Emulsifier, treatment of constipation

Table 4. Correlation of viscosity data with GPC results for methyl cellulose

	A	B
Viscosity Range (cps)	85-115	4000-6000
Mn	131,000	484,000
Mw	369,000	1,023,000
Mz	691,000	1,884,000

There is good correlation between the viscosity data and molecular weight averages.

Calibrants:	Pullulan polysaccharides
Columns:	2 x PL aquagel-OH 60 8 µm, 300 x 7.5 mm (Part No. PL1149-6860)
	1 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent:	0.05M NaH_2PO_4, 0.25M NaCl, pH 7
Flow Rate:	1.0 mL/min
Detector:	RI

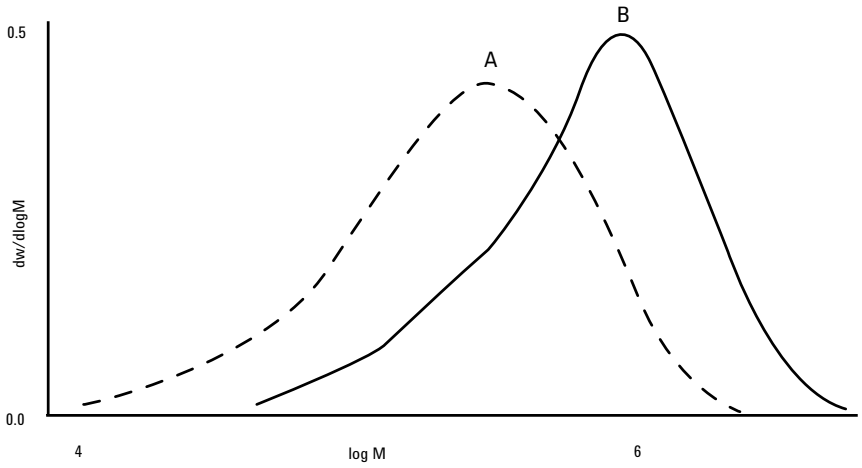


Figure 15. *Example molecular weight distributions for two samples of methyl cellulose which behave very differently*

Cellulose acetate analysis

Application areas: Photographic film base, adhesives, synthetic fibers
Used extensively in the photographic and packaging industries, cellulose acetate is soluble in a limited number of solvents. Here, dissolution was achieved in dimethylacetamide after gentle heating and stirring of the sample solution. Lithium chloride was added to the eluent to counter any polyelectrolyte effects.

Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent:	DMAc+0.5% LiCl
Flow Rate:	1.0 mL/min
Loading:	0.2% w/v, 100 µL
Temp:	60 °C
Detector:	GPC (RI)

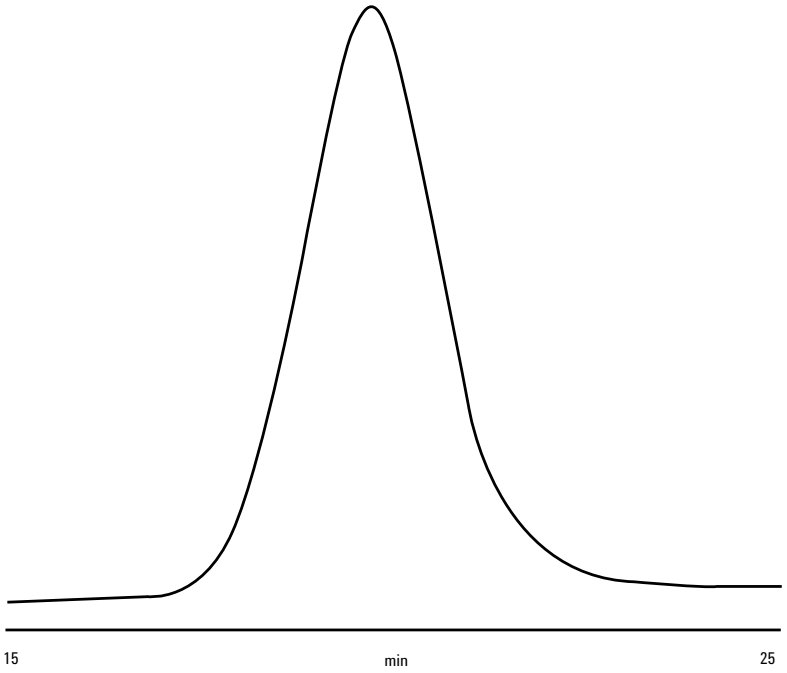


Figure 16. *Example chromatogram for a sample of cellulose acetate analysis showing a typical polymer peak shape*

Pectins

Application area: Gelling agent in food

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 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.

In this application, a sample of pectin was analyzed on the Agilent PL-GPC 50 integrated GPC system running at 30 °C fitted with a refractive index detector, an Agilent PL-BV 400 four capillary bridge viscometer and an Agilent PL-LS 15/90 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 0.2M $NaNO_3$, 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 purpose of light scattering calculations, an average dn/dc value was used for the sample.

Figure 17 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.

Columns: 2 x PL aquagel-OH MIXED 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Detectors: PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

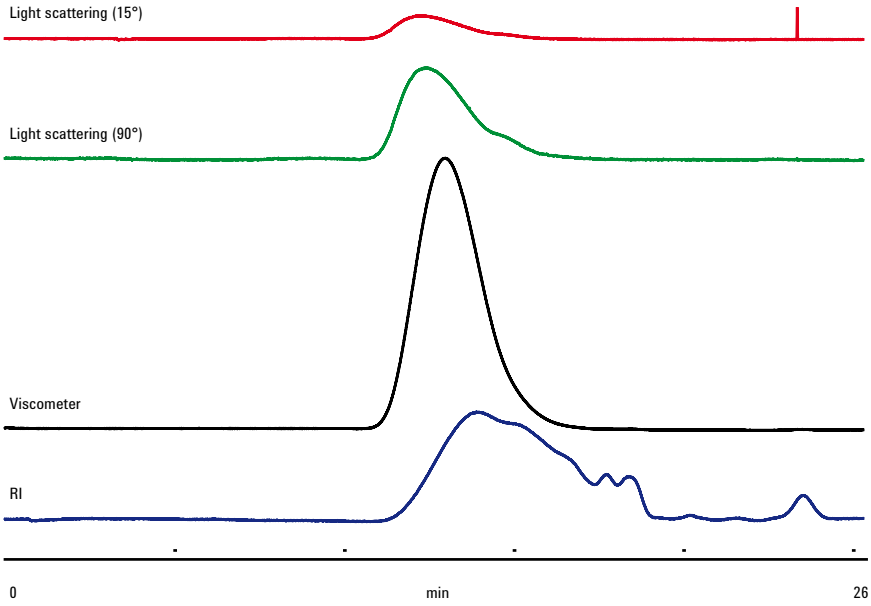
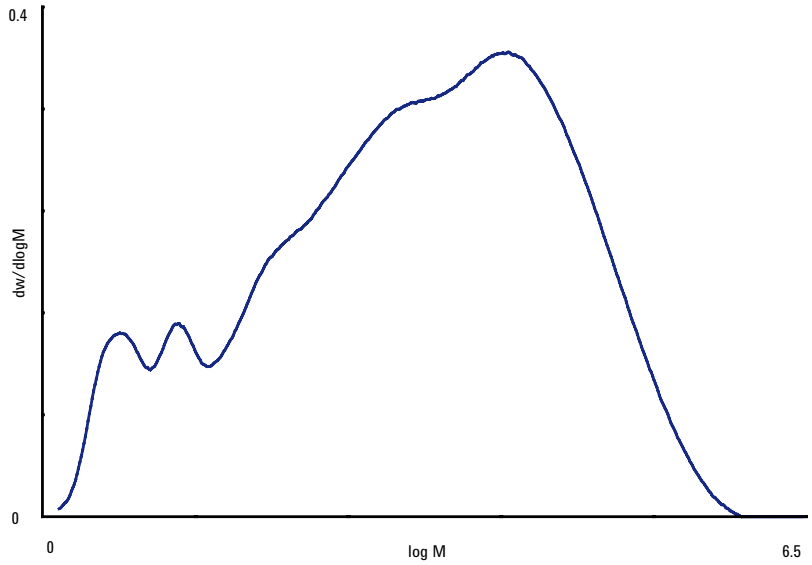


Figure 17. *A typical multi detector overlay of chromatograms for a sample of pectin, showing the different responses of the detector*

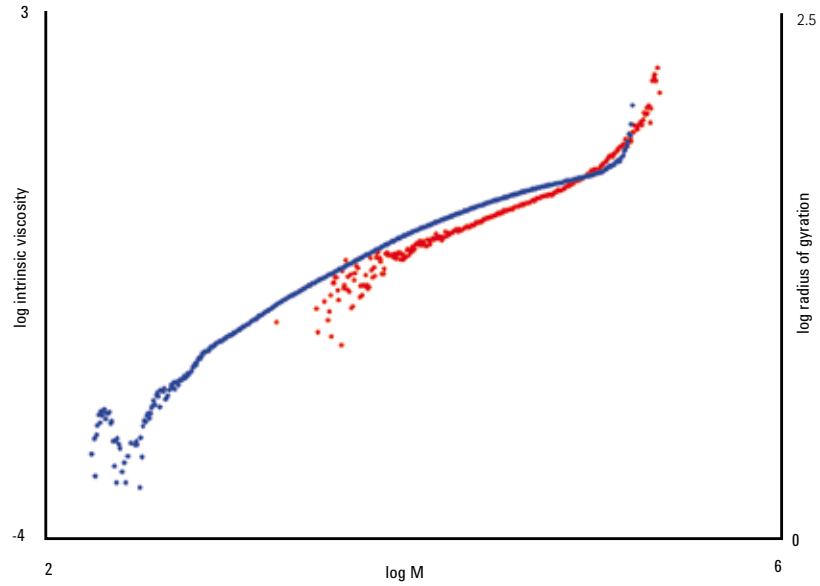
From the viscometry and light scattering data, Mark-Houwink (log intrinsic viscosity versus log M) and conformation (log radius of gyration versus log M) plots were generated for the pectin, shown overlaid in Figure 19.

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. This application demonstrates how the new PL-GPC 50 can be used for the analysis of structurally complex but commercially important materials by multi detector GPC.



Columns:	2 x PL aquagel-OH MIXED 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Detectors:	PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

Figure 18. *Molecular weight distribution calculated for the pectin showing a complex shape*



Columns:	2 x PL aquagel-OH MIXED 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Detectors:	PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

Figure 19. *Mark-Houwink and conformation data showing differences in structure between the two materials*

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Published in UK, October 15, 2010
5990-6920EN

Agilent Technologies