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Plastics and other polymers used during food processing and packaging applications often contain many additives that may significantly influence the final properties of these products. Analysis of the final product is essential to creating a high quality, environmentally safe product. Agilent helps you identify contaminates introduced during processing and offers solutions to determine the point in the manufacturing process that the contamination is occuring.

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Analysis of elastomers by GPC/SEC

Application compendium

Authors

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Agilent Technologies, Inc.

The Measure of Confidence

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Contents Page

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 aquisition 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

Elastomer is a general term used to describe rubbers – polymers that exhibit elasticity. Elasticity is the ability to deform under external stress but return to the original form after removal of the stress. Elastomers may be thermosets that require curing, or thermoplastics that contain both plastic and elastomeric species, and may be natural or synthetic in origin. Thermosetting elastomers are composed of polymeric chains joined by crosslinks, formed by curing reactions such as the vulcanization of natural rubber, creating a loose lattice structure. This allows chains to move relative to one another during deformation but return to their original positions when relaxed, allowing the material to reversibly extend. Without the crosslinkages the applied stress results in a permanent deformation. Thermoplastic elastomers contain plastic and elastomeric regions within the structure, with weaker non-covalent interactions between chains providing the anchor points, allowing the material to return to its original form after removal of the external force.

Gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC) is a well-known technique for assessing the molecular weight distribution of polymers such as rubber, a property that influences many of their physical characteristics as shown in Table 1. In general, increasing molecular weight leads to higher performance, while an increase in the width of the distribution (the polydispersity) leads to a loss of performance but an increase in the ease of processing.

This application compendium describes the analysis of pre-cure thermosetting and thermoplastic elastomers by GPC.

Agilent Technologies, Inc. produces the most extensive range of GPC columns, standards and instruments that are ideally suited to the analysis of synthetic and natural rubber.

Agilent's columns are rugged and reliable, making them ideal for applications that rely on extremely reproducible analysis, such as in quality control environments. With extensive options in particle and pore size, you can select the column to match the molecular weight of the material under investigation, thereby ensuring you get the best quality data from your analysis. To complement our columns we also manufacture narrow polydispersity standards with very highly characterized molecular weights that are used as calibrants in the GPC analysis of rubber polymers.

The quality of our columns and standards is matched by an array of Agilent GPC instruments that cover the widest possible temperature range, from ambient to 220 °C.

These instruments perform all types of GPC/SEC experiments and can be used to analyze the complete range of polymer materials. Multiple detection options may be included in the instruments, such as light scattering and viscometry, or supplied in stand-alone formats, such as the Agilent evaporative light scattering range. Dedicated analysis software allows the biodegradation properties of the materials to be monitored.

The Agilent PL-GPC 50 Integrated GPC/SEC System

GPC/SEC analysis of natural rubbers

The first commercial elastomeric material was natural rubber, derived from the sap of the rubber tree **Hevea brasiliensis**. This substance is composed of a polymer of isoprene, most often cis-1,4-polyisoprene, although some natural rubber sources are composed of trans-1,4-polyisoprene. In the natural unrefined form, isoprene materials are accompanied by small amounts of proteins, fatty acids, resins and inorganic materials. Natural rubber is an elastomer and a thermoplastic. However, reacting the material with sulfur crosslinks the isoprene chains (a process known as vulcanizing), turns the polymer into a thermosetting material. Analysis of a natural rubber by GPC is always of the non-crosslinked, thermoplastic material.

Table 1. Effects of molecular weight distribution on the properties of elastomers

	Strength	Toughness	Brittleness	Melt Viscosity	Chemical Resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

High sensitivity analysis of natural rubber with evaporative light scattering detection

Solutions of natural rubber are generally very difficult to prepare for GPC due to the fact that the polymer contains relatively high levels of 'gel' that are partially crosslinked. Normally, an aliquot of the eluent is added to the weighed sample. This 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. The Agilent 380-ELSD evaporative light scattering detector exhibits significantly increased sensitivity compared to an RI and 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. The 380-ELSD always gives a flat baseline which, together with the improved response, makes baseline and peak setting much more reliable for GPC calculations.

RI is also sensitive to system peaks around total permeation that usually occur even when samples are prepared in an aliquot of the eluent. These system peaks can interfere with low molecular weight components that are commonly found in natural rubber samples. With the 380-ELSD, system peaks are eliminated due to evaporation, leaving unadulterated sample peaks in the additives region.

The Agilent PLgel 10 µm MIXED-B columns, with their high efficiency (>35,000 plates/meter) and broad resolving molecular weight range (up to 10,000,000 daltons relative to polystyrene), are the columns of choice for high molecular weight polymers and demanding eluents. Separation of natural rubber reveals that the combination of PLgel MIXED-B columns with the 380-ELSD comprises a highly sensitive system for the discrimination of additives (Figure 1).

Columns: 3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
Eluent: Toluene
Flow Rate: 1.0 mL/min
Detection: 380-ELSD

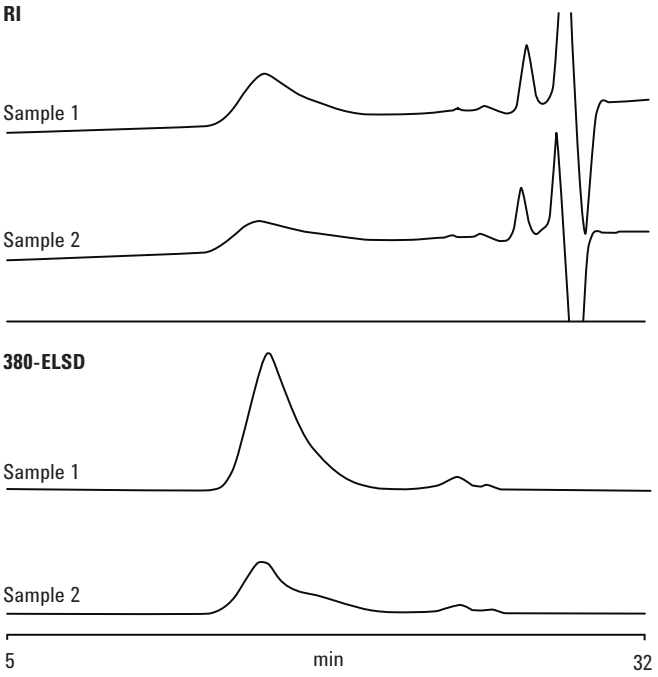


Figure 1. *Stable baseline and no interference from system peaks using the 380-ELSD evaporative light scattering detector (below) compared to RI detection (above) illustrating the advantages of ELSD in this application*

Figure 2 is a magnified view of the additive area revealing the unadulterated peaks in this region of interest.

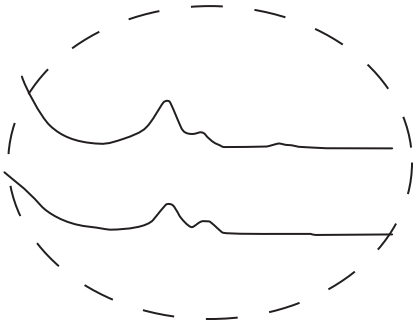


Figure 2. *Magnified view of the 380-ELSD plots showing the additive region - this region is masked with DRI detection, and so ELSD is a better detector for this application*

Analysis of natural rubber with triple detection

Triple detection GPC 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.

Two samples of natural rubber were analyzed by GPC with triple detection. The objective was to determine why one of the materials had failed in end use. An integrated GPC system was used for the analysis.

The samples were analyzed using an Agilent PL-GPC 50 Integrated GPC/SEC System with differential refractive index detector, an Agilent PL-BV 400RT Online Integrated Viscometer, an Agilent PL-RTLS 15/90 Light Scattering Detector, and Agilent PLgel 10 µm MIXED-B columns. These columns provide high resolution of polymers that have high molecular weights, even in demanding eluents.

Figure 3 is a chromatogram of a natural rubber sample showing responses from the different detectors.

Samples:	2 x Natural rubber
Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
Eluent:	Toluene
Injection Volume:	200 µL
Flow Rate:	1.0 mL/min
Temperature:	50 °C
Detection:	PL-GPC 50 with PL-BV 400RT Viscometer and PL-RTLS 15/90 light scattering detector

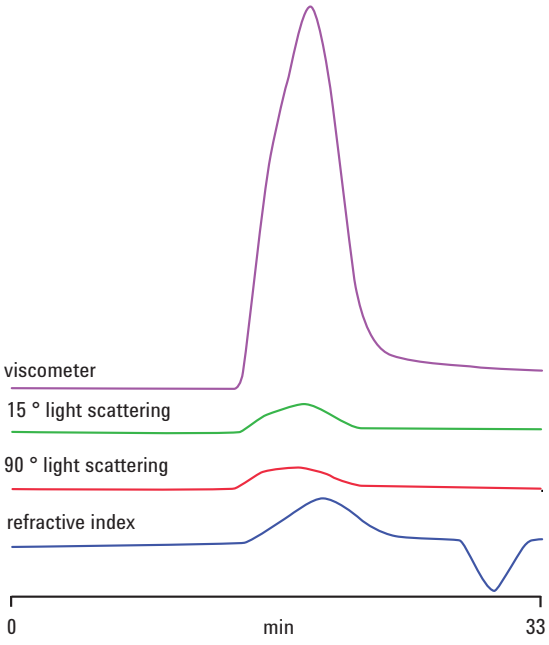


Figure 3. *Raw triple detection data for a natural rubber showing typical peak shapes observed for these materials*

Figure 4 indicates that one of the samples is considerably higher in molecular weight than the other, although the Mark-Houwink plots show that the two materials are structurally similar (Figure 5).

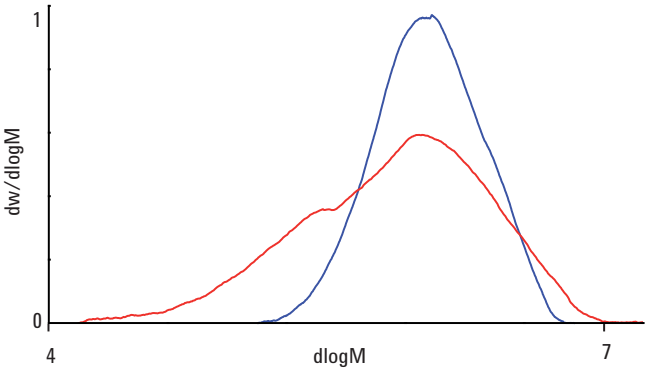


Figure 4. *Overlaid triple detection molecular weight distributions of two natural rubbers with very different distributions and therefore very different final properties*

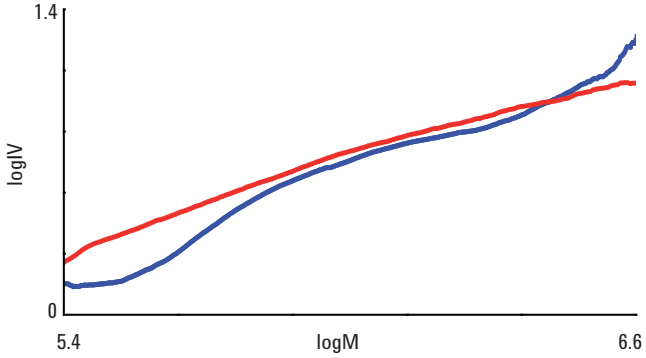


Figure 5. *Overlaid Mark-Houwink plots for two natural rubbers showing the similarity in structure of the materials, with deviation only at low molecular weight*

GPC/SEC analysis of synthetic rubbers

Analysis of synthetic polybutadiene with triple detection

Polybutadiene was one of the first types of synthetic elastomer to be invented and has largely replaced natural rubber in a wide variety of industrial applications.

Two Agilent PLgel 5 µm MIXED-C columns were used for this analysis with the results shown in Figures 6 and 7. The polybutadiene sample was prepared accurately at a nominal concentration of 2 mg/mL in tetrahydrofuran and injected into the system without further treatment. For the purpose of light scattering calculations, an average dn/dc was used for the sample.

Mark-Houwink (log intrinsic viscosity versus log M) plots (Figure 8) were generated from the viscometry and light scattering data. The curvature in the Mark-Houwink plot may be a result of structural changes in the polymer as a function of molecular weight.

Sample:	Polybutadiene
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Eluent:	THF
Injection Volume:	100 µL
Flow Rate:	1.0 mL/min
Detection:	PL-GPC 50 with PL-BV 400RT and PL-RTLS 15/90

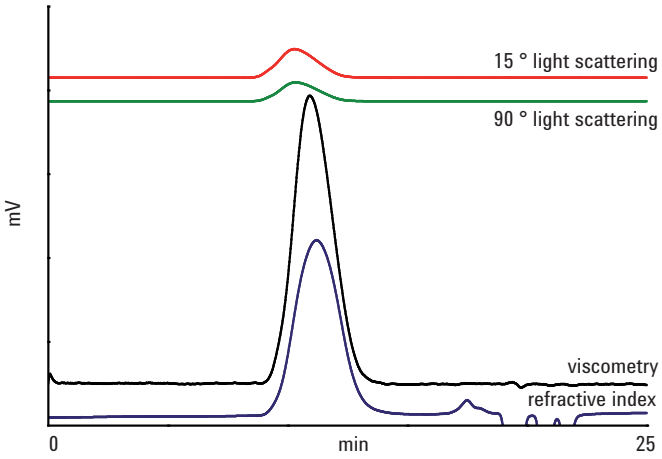


Figure 6. *Triple detection of a polybutadiene showing typical data for this type of sample*

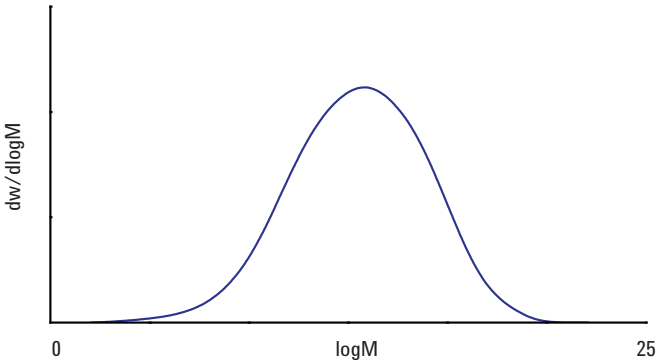


Figure 7. *Molecular weight distribution of a polybutadiene with a broad gaussian peak shape*

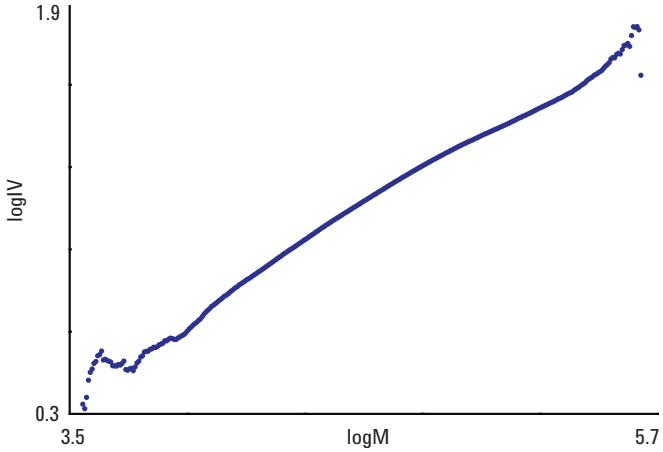


Figure 8. *Mark-Houwink plot of a polybutadiene with curvature that may be attributed to structural changes as a function of molecular weight*

General synthetic elastomer analysis

Polyisoprene can be produced synthetically. Together with polybutadiene and styrene butadiene, it is a common elastomeric material. Polybutadiene is a synthetic rubber manufactured from the monomer 1,3-butadiene. With high wear resistance, it is commonly used in tire manufacture, and to coat electronic assemblies due to its extremely high electrical resistivity. Polybutadiene exhibits 80% recovery after stress, one of the highest stress-recovery values of a synthetic material.

Styrene butadiene rubber (SBR) is a synthetic rubber copolymer of styrene and butadiene. With good abrasion resistance it is widely used in car tires, after blending with natural rubber.

The extended operating range of the PLgel 10 µm MIXED-B column (up to 10,000,000 MW) makes it ideally suited to the analysis of a wide range of high molecular weight elastomers (Figure 9). Sample solutions are routinely filtered prior to injection to remove insoluble "gel fractions", common to most elastomers.

Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
Eluent:	THF
Flow Rate:	1.0 mL/min
Loading:	0.2% w/v, 100 µL
Temperature:	40 °C
Injection Volume:	200 µL
Detection:	Agilent PL-GPC 220

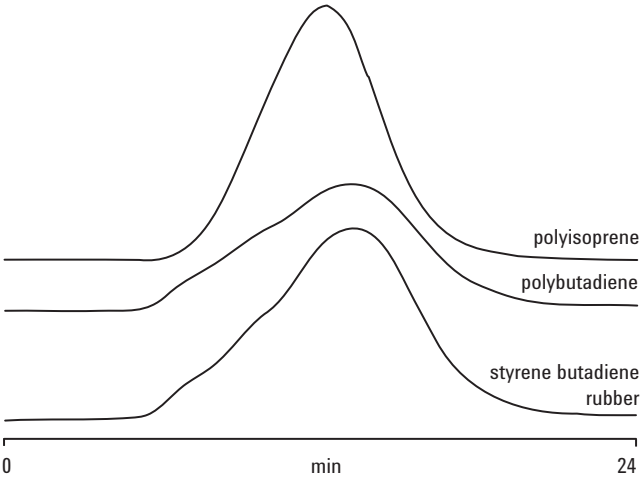


Figure 9. *Chromatograms of three types of synthetic rubber with multimodal peak shapes*

Hexane is a good solvent for butyl rubber although it can be chromatographed using other solvents, such as THF. The polarity of hexane is very low compared with more traditional solvents for GPC such as THF. However, it can be used successfully with PLgel columns (Figure 10).

Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
Eluent:	Hexane
Flow Rate:	1.0 mL/min
Detection:	PL-GPC 50

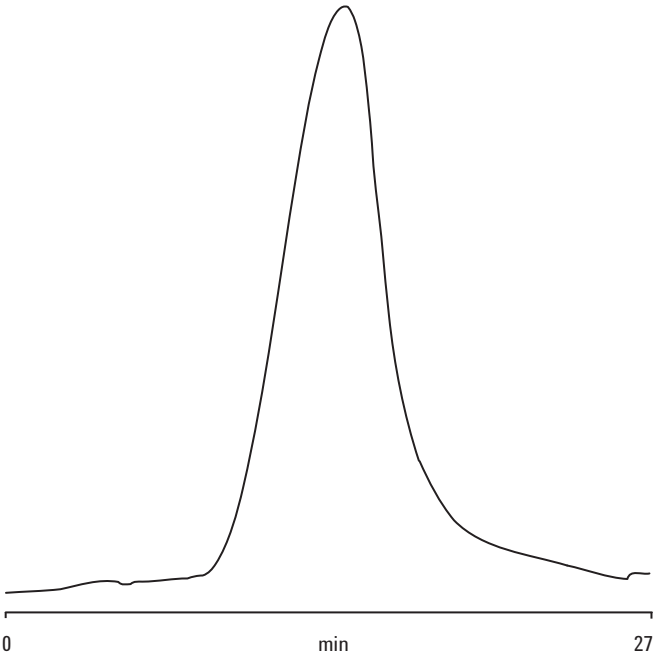


Figure 10. *Butyl rubber chromatogram in hexane showing that a good peak shape can be obtained*

Commercial grades of styrene butadiene rubber can contain very high molecular weight fractions and so, for successful GPC separations, the sample concentration must be minimized in order to avoid viscous streaming effects. Some grades of SBR can also contain low molecular weight mineral oil as a modifier (known as oil-extended grades) that can be resolved from the polymer peak, thus permitting quantification using Agilent ELSD (Figure 11).

Columns:	2 x PLgel 20 µm MiniMIX-A, 250 x 4.6 mm
Eluent:	THF
Flow Rate:	0.3 mL/min
Loading:	1 mg/mL, 100 µL
Detection:	380-ELSD (neb=45 °C, evap=90 °C, gas=0.7 SLM)

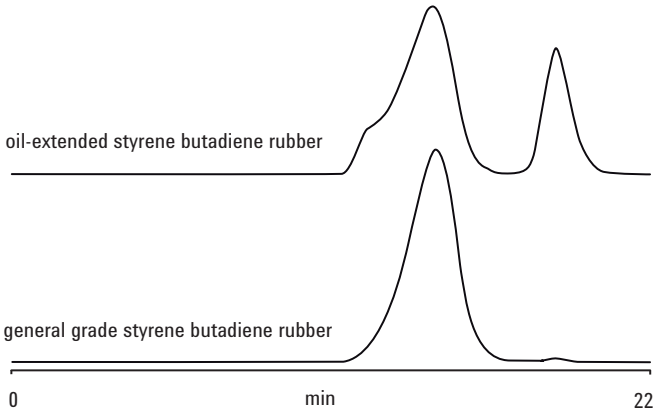


Figure 11. *Chromatograms of two styrene butadiene rubbers, one oil-extended, showing the presence of the low molecular weight additive*

The sample of oil-containing SBR, shown in Figure 12, was analyzed using refractive index detection. To ensure dissolution, the sample was warmed to 50 ºC and gently stirred for three hours. Filtering using 0.5 µm filters is recommended to remove any gel fractions. The PLgel MIXED-B packing permits resolution of both polymer and oil peaks.

Columns:	2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
Eluent:	THF
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Detection:	PL-GPC 50

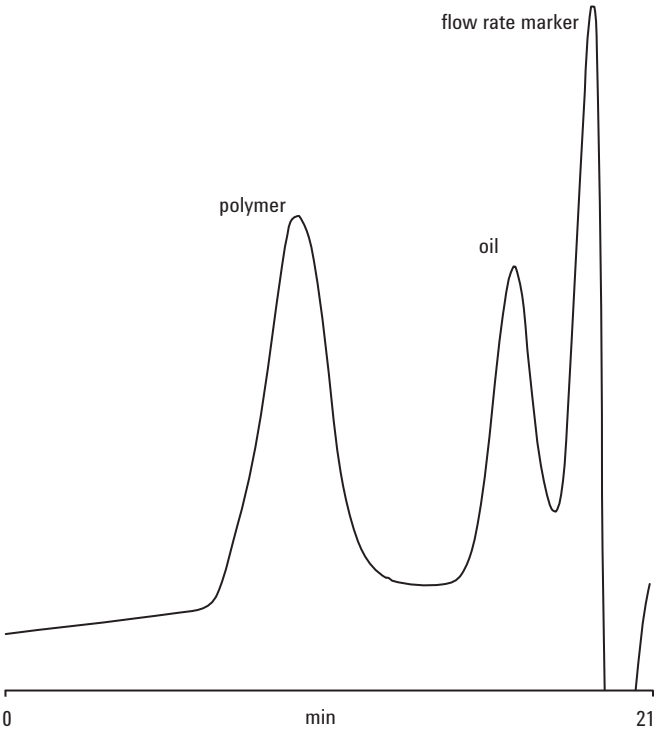


Figure 12. *Chromatogram of an oil-extended styrene butadiene rubber showing the presence of a low molecular weight oil additive*

Polydimethyl siloxane analysis using GPC/ viscometry

Polydimethyl siloxane (PDMS) is a non-toxic, non-flammable silicon-based polymeric material noted for its unusual rheological behavior. Composed of polymer chains of formula $(CH_3)_3SiO[SiO(CH_3)_2]_nSi(CH_3)_3$, PDMS is a viscoelastic material which, with long flow times or at high temperatures behaves like a liquid, and with short flow times or at low temperatures behaves like a rubber. PDMS is produced in a range of grades from liquids through to rubbery semi-solids depending on the molecular weight of the constituent chains. It is a widely used material and can be found in applications such as silicone caulks, lubricants, damping fluids and heat transfer fluids, as well as in breast and knuckle implants. It is also a food additive (E900), used as an anti-foaming and anti-caking agent.

PDMS was analyzed by GPC using the PL-GPC 50 Integrated GPC/SEC System. Due to the importance of the viscometric properties of the material in many final applications, a PL-BV 400RT viscometer was included in the PL-GPC 50 as well as the standard refractive index detector. Results are shown in Figures 13 and 14. This combination of detectors also allows analysis of the material by the Universal Calibration method, giving accurate molecular weights that are not reliant on the chemistry of the standards used for calibration (in this case, polystyrene standards). Although PDMS is soluble in tetrahydrofuran, it is also isorefractive with this solvent and, therefore, THF is not suitable for the analysis and toluene is a more suitable solvent.

Columns:	2 x Agilent PolyPore, 300 x 7.5 mm
Eluent:	Toluene
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Detection:	PL-GPC 50, PL-BV 400RT

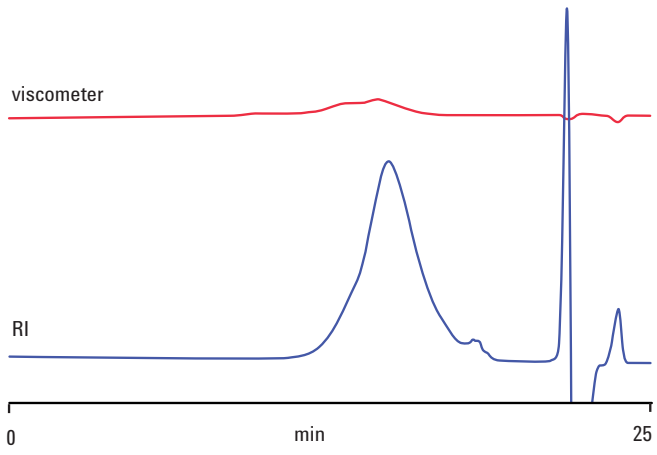


Figure 13. *Example overlaid refractive index and viscometer chromatograms for a sample of polydimethyl siloxane showing typical peak shapes*

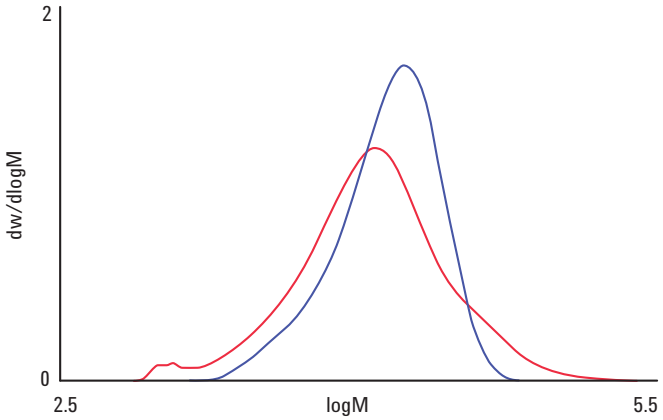


Figure 14. *Overlaid molecular weight distributions for two different grades of polydimethyl siloxane with different performance characteristics*

Although quite different in molecular weight, the Mark-Houwink plot (Figure 15) shows that the two materials are structurally very similar, indicating that their viscoelastic behavior as a function of molecular weight would be comparable.

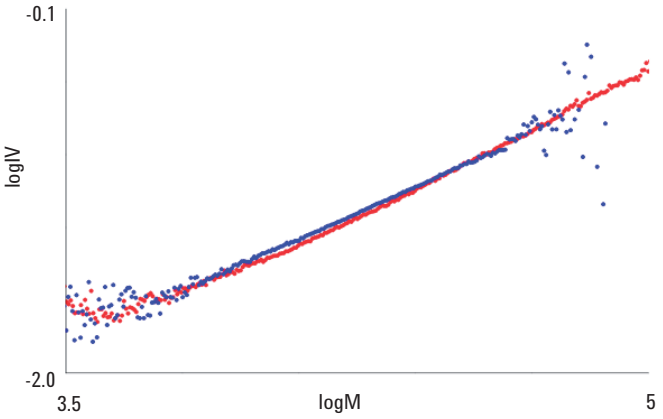


Figure 15. *Mark-Houwink plots calculated for two samples of polydimethyl siloxane show that the materials are structurally very similar*

Analysis of poly(styrene/butadiene) copolymers by conventional GPC

A poly(styrene/butadiene) block copolymer mimics many of the properties of natural rubber and has applications in a wide variety of industrial areas. Its characteristics are provided by the hard polystyrene chains being surrounded by a network of rubbery polybutadiene, which provides strength and flexibility over a large temperature range. The copolymer is a thermoplastic elastomer and therefore can be easily used in manufacturing by injection molding, or blended into an existing product to increase elasticity or impart toughness. The molecular weight distribution is critical, as any homopolymer will significantly affect the resultant end properties.

In the analysis described here distinct differences were observed arising from the presence of homopolymers along with the intended copolymer (Figures 16 and 17).

Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Calibration Standards:	Polystyrene EasiVial
Eluent:	THF (250 ppm BHT)
Temperature:	40 °C
Injection Volume:	100 µL
Detection:	PL-GPC 50

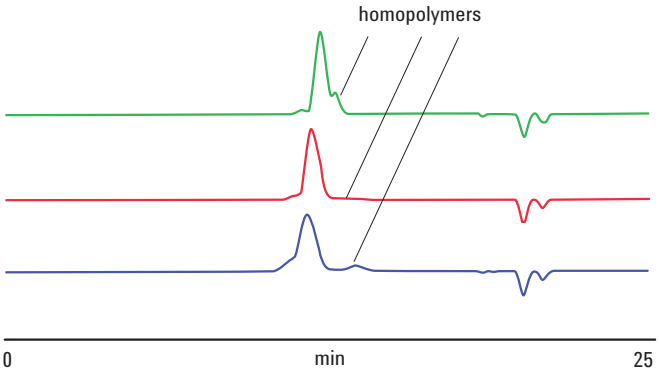


Figure 16. *Chromatograms for styrene butadiene rubbers showing the presence of homo- and copolymers*

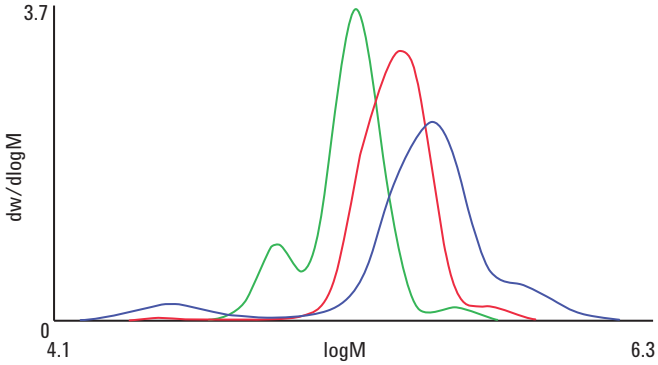


Figure 17. *Overlaid molecular weight distributions for the styrene butadiene rubber samples showing a clear change in molecular weight distribution between samples*

More Agilent solutions for the analysis of rubber

Although well-suited to the analysis of natural and synthetic rubber, GPC is not the only analytical technique available for the examination of these important materials. Agilent offers a range of instruments and consumables that can be used to test elastomers in environmental protection, recycling schemes, confirmation of equivalents, deformulation, quality control and failure analysis. Agilent instruments are particularly valuable for the detection of additives or modifiers, ubiquitous components found in most commercially useful rubbers.

Spectroscopy

Both the AA and ICP-OES techniques are suitable for determining the "filler" and trace levels of metals in compounded rubber products. For toys made from rubber materials, there are worldwide standards applicable that require the determination of a range of toxic elements.

For example, the US Consumer Product Safety Improvement Act of 2008 (CPSIA), EN 71 Part 3 and AS/NZS ISO 8124.3:2003 standards define requirements for testing the migration of inorganic elements from toys and children's products. Agilent AA and ICP-OES instruments meet and exceed these testing requirements. Analysts may also use AA and ICP-OES for monitoring and evaluating the environmental impact of waste rubber products (especially used tires) in land fill sites, artificial reefs, or assessing trace element levels in fly ash from tire-derived fuel.

NMR

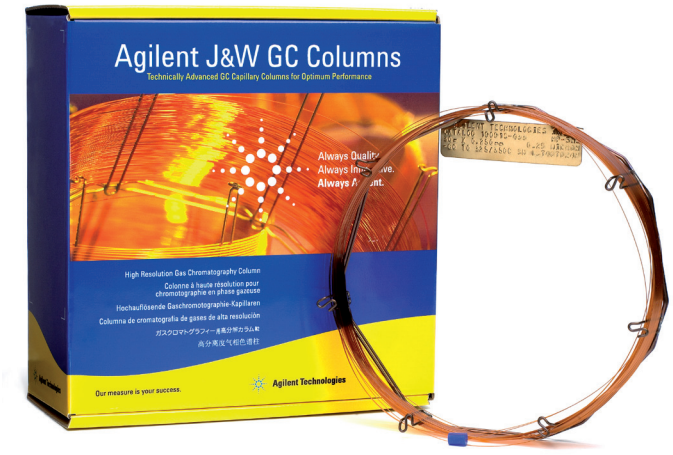
Rubber NMR is a well-established subfield of Solids NMR. MAS (magic angle spinning) is used to improve shimming, and rubber spectra could be run in either a Solids MAS probe or a Nanoprobe. More advanced experiments that measure relaxation properties are employed to analyze dynamics and morphology, such as domain sizes, and the results have been used to explain the dielectric and mechanical properties of rubber. Agilent's 400, 500 or 600 MHz NMR systems are ideal for such tasks when equipped with accessories for solid state NMR.

The Agilent 400-MR Magnetic Resonance Spectrometer is the system of choice for laboratories requiring a compact and easy-to-use instrument that delivers fast and reliable results day in and day out

GC

Toxic gases that are emitted during the processing and curing of rubber goods can be monitored using Agilent gas chromatography. The same technique helps ensure product safety when determining levels of plasticizers or nitrosamines in food rubbers, such as dummies for babies or teats for baby bottles. For the two ASTM rubber methods, we recommend Agilent J&W CP-PoraBOND Q GC columns to detect acrylonitrile in nitrile rubber.

The Agilent 7890A GC with 7693A Series Automatic Liquid Sampler



Agilent J&W offers the largest selection of the highest quality GC columns available today

FTIR

FTIR spectroscopy has a powerful ability to provide both chemical and structural information of a sample. Rapid, non-destructive, chemical identification is possible with easy-to-use Attenuated Total Reflectance (ATR) accessories. Agilent also provides thermally controlled accessories that enable users to investigate their elastomer sample under their desired environmental conditions. Or, if combined with thermogravimetric analysis (TGA), Agilent's hyphenated TGA/FTIR analyzer delivers real-time monitoring of gases evolved from rubber samples, and enables complex chemistries to be unravelled and understood. Our TGA/FTIR systems consist of an FTIR spectrometer and TGA/IR interface accessory with data interpretation performed using powerful Resolutions Pro software.

The Agilent 600 Series FTIR provides the highest level of sensitivity combined with detailed structural and compositional information for information-rich detection

Ordering information

Agilent offers robust instrumentation, application-based consumables, and customer-focused services, backed by our global team of product and applications experts, ready to help you solve your analytical challenges. Whether you're monitoring impurities in drinking water, designing new therapeutic drugs or developing cleaner fuels, our solutions deliver the sensitivity, flexibility and productivity your laboratory requires.

Columns

Description	Part No.
Agilent PLgel 5 µm MIXED-C, 300 x 7.5mm	PL1110-6500
Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm	PL1110-6100
Agilent PLgel 20 µm MiniMIX-A, 250 x 4.6 mm	PL1510-5300
Agilent PolyPore, 300 x 7.5 mm	PL1113-6500
Agilent EasiVial PS-H (2 mL)	PL2010-0201
Agilent EasiVial PS-H (4 mL)	PL2010-0200
Agilent EasiVial PS-L (2 mL)	PL2010-0401
Agilent EasiVial PS-L (4 mL)	PL2010-0400
Agilent EasiVial PS-M (2 mL)	PL2010-0301
Agilent EasiVial PS-M (4 mL	PL2010-0300
Agilent PS-H 2 mL Tri-Pack (90 Vials)	PL2010-0202
Agilent PS-H 4 mL Tri-Pack (90 Vials)	PL2010-0203
Agilent PS-L 2 mL Tri-Pack (90 Vials)	PL2010-0402
Agilent PS-L 4 mL Tri-Pack (90 Vials)	PL2010-0403
Agilent PS-M 2 mL Tri-Pack (90 Vials)	PL2010-0302
Agilent PS-M 2 mL Tri-Pack (90 Vials)	PL2010-0303

Standards

Description	Part No.
Agilent EasiVial PS-H (2 mL)	PL2010-0201
Agilent EasiVial PS-H (4 mL)	PL2010-0200
Agilent EasiVial PS-L (2 mL)	PL2010-0401
Agilent EasiVial PS-L (4 mL)	PL2010-0400
Agilent EasiVial PS-M (2 mL)	PL2010-0301
Agilent EasiVial PS-M (4 mL	PL2010-0300
Agilent PS-H 2 mL Tri-Pack (90 Vials)	PL2010-0202
Agilent PS-H 4 mL Tri-Pack (90 Vials)	PL2010-0203
Agilent PS-L 2 mL Tri-Pack (90 Vials)	PL2010-0402
Agilent PS-L 4 mL Tri-Pack (90 Vials)	PL2010-0403
Agilent PS-M 2 mL Tri-Pack (90 Vials)	PL2010-0302
Agilent PS-M 2 mL Tri-Pack (90 Vials)	PL2010-0303

Instruments

Description	Part No.
Agilent PL-GPC 220 Integrated GPC/SEC System	PL0820-0000
Agilent PL-GPC 50 Integrated GPC/SEC System	PL0870-8500
Agilent PL-BV 400HT Online Integrated Viscometer	PL0810-3050
Agilent PL-BV 400RT Online Integrated Viscometer	PL0810-3060
Agilent PL-HTLS 15/90 Light Scattering Detector for PL-GPC 220	PL0640-1200
Agilent PL-RTLS 15/90 Light Scattering Detector for PL-GPC 50	PL0640-1210
Agilent 380-LC Evaporative Light Scattering Detector (110 V)	PL0890-0110
Agilent 380-LC Evaporative Light Scattering Detector (240 V)	PL0890-0240

For full ordering information, visit
www.agilent.com/chem/store

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
Biodegradable polymers	5990-6920EN
Engineering polymers	5990-6970EN
Polyolefin analysis	5990-6971EN
Low molecular weight resins	5990-6845EN

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Agilent Technologies

Analysis of polyolefins by GPC/SEC

Application Compendium

Authors

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Agilent Technologies, Inc.

The Measure of Confidence

Agilent Technologies

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Polyolefin analysis by GPC/SEC

Introduction

Polyolefin is a general term describing polymers created from simple olefins or alkenes. Many different types of olefin exist, from the most simple, ethylene, to alpha-olefins of increasing complexity. Polyolefins are of great interest as two of them, polyethylene (polythene) and polypropylene, are among the highest tonnage polymers produced in the world. Interest in the analysis of polyolefins comes from the desire to create new materials with custom properties, from the development of new catalysts and from the need to perform quality control on polymer production.

Agilent has a long history of involvement in the analysis of polyolefins by gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC). This application booklet describes Agilent's product portfolio for polyolefin analysis. Instrumentation, software, columns and standards are described, providing a complete package for the analysis of these important products. In addition, a wide range of applications are included that illustrate the performance of the complete solutions for polyolefin analysis offered by Agilent.

Gel permeation chromatography is a well-known technique for assessing the molecular weight distribution of polymers such as polyolefins. Molecular weight influences many of their physical characteristics, as shown in Table 1. In general, increasing molecular weight leads to higher performance, while an increase in the width of the distribution (the polydispersity) leads to a loss of performance but an increase in the ease of processing.

Many polyolefins, typically those containing over 10% ethylene and polypropylene monomers, are of limited solubility in a number of solvents. This is because the characteristic high strength and toughness of these materials results from their high crystallinity. Increased crystallinity requires break up of any inter-chain bonds in order to dissolve the material. Several solvents can be used, but in general the most effective is trichlorobenzene, a viscous solvent with a distinct odor. Ortho-dichlorobenzene is also used in some laboratories, but solubility in this solvent is less effective.

Table 1. *Effects of molecular weight (Mw) and the impact of decreasing the width of distributon of Mw on polyolefins*

	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

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.

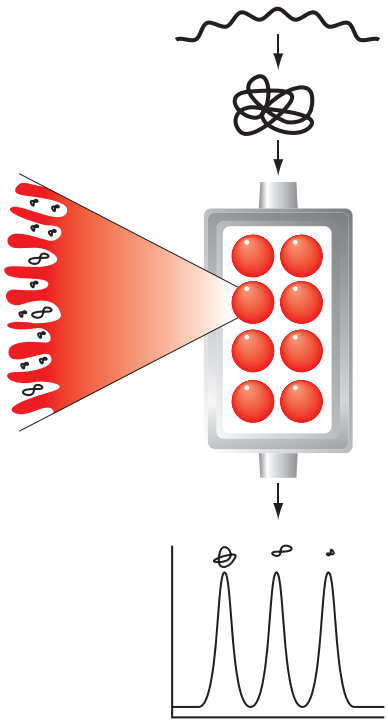
With the acquisition of PL, Agilent offers an even wider range of GPC and SEC solutions for all types of polymer characterization of synthetic and bio-molecular polymers, with options for conventional GPC all the way up to complex determinations using multi-column and multi-detection methods.

The GPC separation mechanism

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- Polymer coils introduced to eluent flowing through a column
- Column packed with insoluble porous beads with well-defined pore structure
- Size of pores similar to that of polymer coils
- Polymer coils diffuse in and out of the pores
- Result is elution based upon size – large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards

Highly crystalline polymers such as polyethylene are soluble only at high temperatures. This is because elevated temperatures are required to break down the ordered crystalline structure, and on cooling the material will re-crystallize and precipitate from solution. For these applications, high temperature is required throughout the entire analysis to ensure that the samples remain in solution. This places several requirements on the instrument for the successful analysis of polyolefins.

- Solvent choice is limited, mainly to 1,2,4-trichlorobenzene (TCB)
- Elevated temperature is required for dissolution, typically for 1 to 4 hours depending on molecular weight and crystallinity
- Column selection must be appropriate for the application in terms of molecular weight resolving range and efficiency of separation
- A high temperature GPC system is required to maintain all components at the analysis temperature, typically 135 to 170 °C, depending on molecular weight and crystallinity



Key

Smaller coils can access many pores

Larger coils can access few pores

Very large coils access very few pores

GPC system requirements for polyolefin analysis

Autosampler, detectors, columns, injection valve and transfer tubing must all be capable of handling elevated temperatures during polyolefin analysis. A typical system schematic is shown in Figure 1.

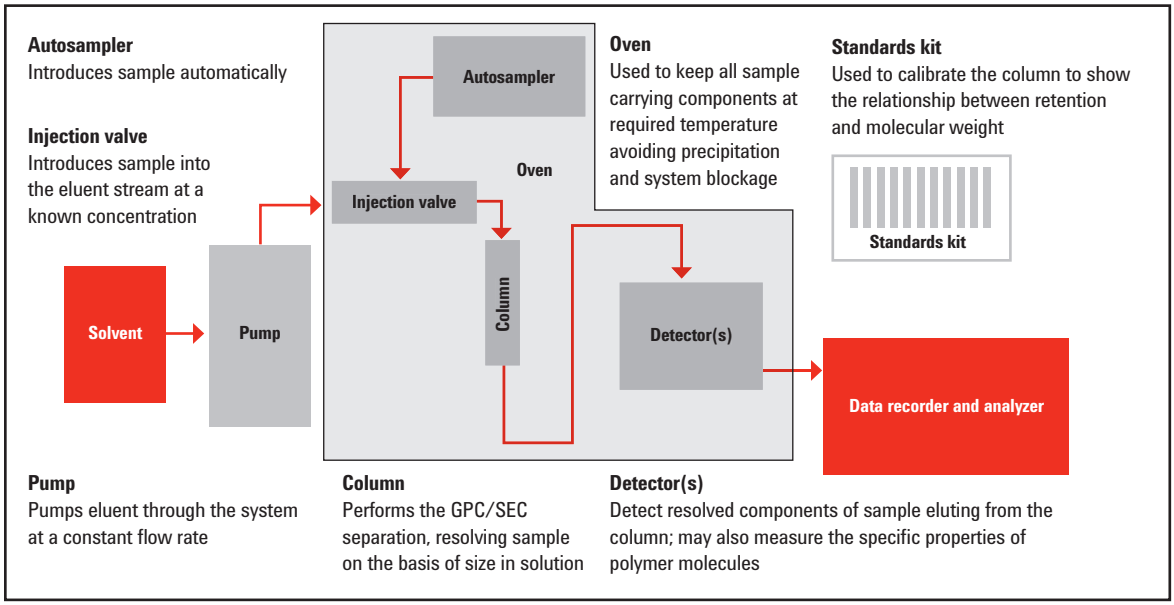


Figure 1. *Schematic of a GPC system for polyolefin analysis*

Sample preparation

Preparing polyolefin samples is time-consuming because high temperatures and long heating times are required to dissolve the sample (Table 2). Many polyolefins also display a lower density than common analytical solvents such as TCB, and so agitation of the sample is essential to ensure complete dissolution. Filtration may also be necessary to remove insoluble material such as fillers.

Table 2. *Preparing a polyolefin sample for analysis*

Material	Typical concentration (mg/mL)	Typical prep temp (°C)	Typical heating time (h)
Olefin wax	2 to 3	150	1
General PE or PP	2	150	4
Ultra-high-molecular-weight polyolefin	0.25 to 0.5	150	4 to 8

Agilent PL-SP 260VS Sample Preparation System

The PL-SP 260VS is designed for the manual dissolution and filtration of samples such as polyolefins prior to GPC analysis. The unit combines controlled heating across a temperature range of 30 to 260 °C (± 2 °C), with gentle agitation, user-selectable between 85 to 230 (± 10%) rpm. With its temperature range and speed capabilities, the PL-SP 260VS is ideal for a wide range of polymer types, including even the most difficult of samples such as ultra-high-molecular-weight polyethylene.

Choice of vial types

The removable aluminium blocks for the heated compartment are available in several formats to accommodate a variety of vial types. The Standard Accessory Kit is used with standard sample preparation 20 mL vials (supplied) and either PL-GPC 220 2 mL autosampler vials or 4 mL autosampler vials from other vendors. The Custom Accessory Kits let you choose alternative vials, if necessary.

Agilent PL-SP 260VS Sample Preparation System

Efficient dispensing

A unique pipettor device efficiently dispenses filtered sample solution from the sample preparation vial directly into destination (autosampler) vials with minimal handling.

Choice of filtration media

Filtration of polyolefin samples is often required to remove insoluble fillers or gel content (Figure 2). Two filter media are available:

- Glass-fiber (nominal porosity 1 µm) – the preferred system for general applications (Figure 2)
- Porous stainless steel (nominal porosity 0.5, 5, and 10 µm)

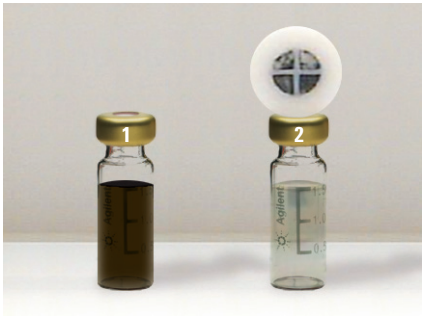


Figure 2. *Filtering a carbon black polyethylene solution – 1. without filtration, 2. after filtration using a 1 µm glass-fiber filter*

System, software and standards

The Agilent PL-GPC 220 Integrated GPC/SEC System for polyolefin analysis

The PL-GPC 220 is a leading system for the analysis of polyolefins at high temperature. Containing a number of features that have been specifically designed for polyolefin analysis, the PL-GPC 220 is the most versatile instrument for gel permeation chromatography.

Widest temperature range

The PL-GPC 220 features the widest operating range available: 30 to 220 °C, permitting analysis of virtually any polymer in any solvent. The multi-heater, forced-air oven is extremely stable, and accurately controls the temperature to within 0.05 °C. This minimizes detector baseline drift, ensuring the reproducible retention times so important in GPC.

High-precision isocratic pump – unrivalled reproducibility for precise results

The PL-GPC 220 incorporates a high-precision pump for the best pump performance available. Unbeatable flow reproducibility of 0.07% is achieved, not only in THF at near-ambient temperature, but also in TCB at temperatures above 140 °C.

Easy-access oven – changing columns and routine maintenance made simple

The column oven can comfortably hold six, 300 x 7.5 mm GPC columns. The oven operates at a convenient angle to allow for easy access for changing columns and the injector loop, providing comfortable and safe operation.

Agilent PL-GPC 220 Integrated GPC/SEC System

Enhanced RI sensitivity and stability

The improved refractive index (RI) detector includes a new photodiode and uses fiber optic technology to maximize sensitivity while minimizing baseline drift and noise, vital for good GPC/SEC. This RI detector delivers outstanding signal-to-noise ratios, even at 220 °C (Figure 3).

Conditions

Columns: 2 x Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Flow Rate: 1 mL/min
Inj Vol: 200 µL
Detector: PL-GPC 220

Peak Identification

1. Mp = 1,460,000, conc. = 0.62 mg/mL
2. Mp = 9,860, conc. = 1.08 mg/mL

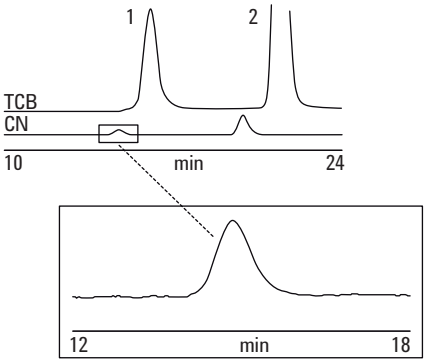


Figure 3. *Excellent signal-to-noise demonstrated in the separation of polystyrene standards*

Safety first – solvent leak detection and automated shutdown

Agilent's GPC/SEC systems incorporate integral sensors that constantly monitor the system. Vapor sensors are fitted in both the solvent module and column oven. The sensors can be programmed for sensitivity according to the solvent in use.

In the case of an unattended error, the system selects and activates the appropriate shutdown sequence depending on the nature of the error. Low solvent flow will be maintained, where possible, to avoid damage to valuable GPC columns.

An audit trail feature offers full status and error logging for system traceability.

Customized upgrade solutions

The oven easily handles multiple-detector upgrades such as light scattering and viscometry, and coupling to other techniques such as TREF (temperature rising elution fractionation), FTIR (fourier transform-infrared spectroscopy) and ELSD (evaporative light scattering detection). The oven holds up to four detectors in combination. For example, integrating RI, viscometry and light scattering would provide complete polymer characterization.

PC control – easy to program, easy to use

The PL-GPC 220 system for polymer characterization up to 220 °C features intuitive, comprehensive PC software control for full and flexible system management. With safety a pre-requisite, PC control uniquely permits remote use so that you do not need to be in the laboratory.

Interactive color-coded graphics provide ease-of-use. Simply click on the color-coded modules via the main screen to alter any run parameters. Flow rate, temperature and autosampler sequence are quickly and easily updated, and on-screen help is always available, if required (Figure 4).

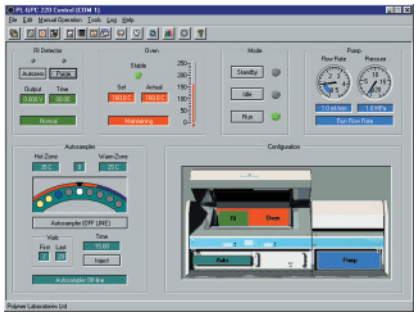


Figure 4. *Software control of the PL-GPC 220*

The time estimator calculates the amount of solvent you will need to run your samples. Input the day and time you want the system to start, then load your samples into the autosampler and let the PL-GPC 220 take care of the analysis for you.

The PL-GPC 220 is designed for true unattended operation. The system gradually heats to the analysis temperature, while the pump maintains a low flow of solvent through the column set. Once temperature is reached and stable, the pump ramps gradually to the flow rate required to run your sample. The PL-GPC 220 then automatically purges the RI detector and autozeros the baseline. Detector output is monitored and when stable, the autosampler loads and injects the first sample. Once the run sequence is complete, the flow rate automatically reduces to conserve solvent.

Integrated solvent delivery – safety by design

The solvent module in the PL-GPC 220 provides a safe, controlled environment in which solvent and waste are managed. Solvent handling is fully integrated and vented for operator safety, and the system does not need to be located in a fume hood.

The PL-GPC 220 includes an integral solvent degasser with a choice of solvent reservoir from 2 L bottles up to a 13 L stainless-steel tank. The solvent delivery module is thermostatically controlled to 30 °C, which ensures efficient, continuous and reproducible solvent delivery, even if the solvent is viscous or may be solid at near-ambient temperature (Figure 5).

Figure 5. *Agilent PL-GPC 220 integrated solvent delivery system*

Dual-zone-heated autosampler – no degradation of samples before injection

Agilent's innovative autosampler accommodates 39 samples in industry-standard 2 mL vials. Injection precision has been measured at better than 1% RSD with no cross contamination between samples, and without the need for rinse vials. The autosampler design features dual-zone heating to minimize thermal degradation. The warm and hot zones are independently programmable from ambient to 220 °C, and so the samples in the carousel waiting for injection are maintained at a lower holding temperature, then heated to analysis temperature prior to injection.

The vial is transferred to the column oven where the sample equilibrates before injection. This minimizes baseline disturbance and completely eliminates the risk of sample precipitation.

Agilent Cirrus GPC Software – the universal GPC solution

Cirrus is the powerful suite of GPC/multi-detector software from Agilent. Polymer Laboratories, now a part of Agilent, has been a supplier of industry-standard GPC software since the 1980s. Cirrus makes GPC calculations easy, whether in conventional GPC using a concentration detector or for multi-detector analysis with light scattering and viscosity.

Integration with existing LC software

Powerful, yet easy to use and learn, Cirrus is available for standalone GPC or for integrating GPC with LC. Cirrus utilizes the latest advances in software design to provide comprehensive calculation options, customized reporting, and high-resolution data capture with the Agilent PL DataStream.

Modular, flexible, and scalable

Cirrus is made to grow as your needs change. A suite of modules provides support for a variety of GPC techniques, such as multi-detector GPC, online FTIR detection and short-chain branching (SCB). Cirrus can be run on a standalone PC or provide a networked GPC solution.

Easy-to-use interface

Cirrus uses an intuitive graphical-user interface, so straightforward that new users can report results within an hour of installing the software. Cirrus is based on Agilent's Workbook concept to provide:

- A simple 'container' for data, parameters and results
- Automatic archiving of chromatograms, calibrations, and results
- Data traceability and data integrity
- Templates allowing predefinition of parameters and report content

Comprehensive calibration and calculation options

Cirrus offers a choice of calibration options.

- Conventional calibration using narrow standards
- Universal Calibration by viscometry or using Mark-Houwink coefficients
- Replicate entries of calibration points
- Three broad-standard calibration methods
- Averages and distributions can be calculated for any number of peaks in a chromatogram
- % of material can be reported for specific MW limits

A calibration overlay facility lets you view the effects of column performance over time.

Reviewing, collating, and condensing results

Cirrus meets the requirements of both QC/Routine and R&D environments, providing fully automated or interactive analysis. The software offers a number of powerful options to review, compare and extract information from archived data and results for inclusion into final reports.

Chromatograms and results can be reviewed both textually and graphically. This information can be exported in a variety of industry-standard formats. A powerful report designer provides total flexibility in report content and presentation. In Cirrus, all parameters relating to a chromatogram or results file are easily accessible via a comprehensive range of export options. Cirrus also ensures that data integrity and traceability are maintained throughout all operations.

Standards for column calibration in polyolefin analysis

Polymer standards from Agilent Technologies are the ideal reference materials for generating accurate, reliable GPC/SEC column calibrations, with the assurance of the ISO 9001:2000 quality standard. Additional applications for our highly characterized homopolymers exhibiting unique characteristics are as model polymers for research and analytical method development. These quality polymer standards are supplied with extensive characterization that utilize a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign that all important peak molecular weight (Mp).

For polyolefin analysis, polyethylene and polystyrene standards are commonly employed. Agilent provides you with the widest choice of these materials to maximize your specific characterization needs. In addition, we supply other polymers as individual molecular weights, and broad distribution polymers for system validation or broad standard calibration procedures. A range of polymer standards available from Agilent are listed in Table 3.

Table 3. *Standards selection guide*

Polymer type	Individual Mw	Calibration kits	Agilent EasiCal	Agilent EasiVial	Type of GPC/SEC
Polystyrene	Yes	Yes	Yes	Yes	Organic
Polymethylmethacrylate	Yes	Yes		Yes	Organic
Polyethylene	Yes	Yes			Organic

Recommendations for setting up a GPC/SEC system for polyolefin analysis

The following questions will help you find the recommended columns and standards for any given application, as well as the system parameters such as injection volumes.

Choosing a column for GPC/SEC of polyolefins

Columns shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
1. What is the expected molecular weight? *It may seem strange to ask this question, but in GPC/SEC the resolution of a column is related to the resolving range. Knowing something of the expected molecular weight of a sample helps to choose the best column that will give optimum results.*	High (up to several millions)	**PLgel Olexis**	PLgel Olexis is specifically designed for polyolefin analysis, offers optimal performance, also suitable for light scattering
		PLgel 10 µm MIXED-B or PLgel 20 µm MIXED-A	The PLgel MIXED-A column resolves higher than the PLgel MIXED-B but at lower efficiency due to larger particle size
		PLgel MIXED-B LS or PLgel MIXED-A LS	Suitable for light scattering
	Intermediate (up to hundreds of thousands)	PLgel 5 µm MIXED-C or PLgel 5 µm MIXED-D	These PLgel columns are the most widely applicable for the majority of applications
	Low (up to tens of thousands)	**PLgel 5 µm 500Å**	The PLgel column provides high resolution and is designed for low-molecular-weight applications
	Very low (a few thousand)	**PLgel 5 µm 100Å**	The PLgel column gives high resolution at low Mw
	Unknown	**PLgel Olexis**	This PLgel column is designed for polyolefin analysis
2. How many columns to use? *The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution and the more columns are required to maintain the quality of the results. For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples during analysis.*	Depends on the particle size of the columns	Particle size 20 µm, use 4 columns	Increased number of columns required for large particle sizes to make up for low efficiences – PLgel Olexis is 13 µm
		Particle size 13 µm, use 3 columns	
		Particle size 10 µm, use 3 columns	
		Particle size 5 µm, use 2 columns	
3. What standard is best? *Depending on analysis there are two options.*		**Polystyrene (PS)** or polyethylene (PE)	Polystyrene is the most commonly used standard in convenient EasiVial format, polyethylene is useful for generating PE based molecular weights

Columns for GPC analysis of polyolefins

Agilent produces a broad array of columns for the analysis of synthetic polymers and many of them are suitable for the analysis of polyolefins. However, the PLgel Olexis column is specifically designed for polyolefins with a wide range of molecular weights.

Agilent PLgel Olexis

PLgel Olexis is the optimum column choice for the analysis of very high-molecular-weight polymers such as polyolefins. Designed and manufactured specifically for these compounds, the column resolves up to 100,000,000 g/mol (polystyrene in THF). Packed with 13 µm particles for maximum resolution with minimal polymer shear, the columns also operate up to 220 °C for the analysis of highly crystalline materials. The column packing exhibits the excellent mechanical stability and robustness expected from the PLgel product range.

No shear degradation

The columns have a particle size of 13 µm, selected to give good efficiency in excess of 30,000 plates/m. In addition, the excellent size consistency of the particles (Figure 6) results in a very narrow particle size distribution that ensures no shear degradation.

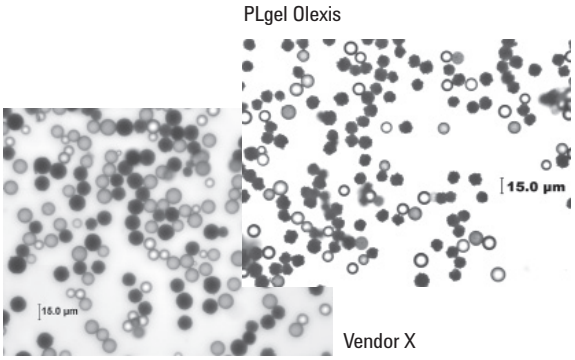


Figure 6. *The superior size consistency of PLgel Olexis particles is clearly evident*

High resolving range

Many new types of polyolefins have been developed recently with very high polydispersities. Determination of accurate polydispersities and modalities is critical in the research and development of these new polymers. PLgel Olexis completely satisfies this demand, for all polyolefin applications up to 100,000,000 g/mol.

Easy extrapolation

The large pore size of the particles makes them effective with many types of polyolefin. Linearity was introduced into the Agilent manufacturing process as a control criterion to ensure linear resolution across the operating range (Figure 7). The result is simplified extrapolation for calibrations.

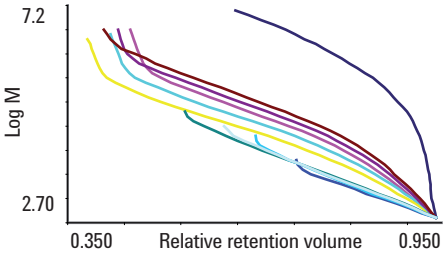


Figure 7. *Some of the components of PLgel Olexis that contribute to its lack of artifacts*

One column for all polyolefin applications

As the packing material in PLgel Olexis is an accurate blend of many components, smooth distributions are produced that truly reflect the sample composition (Figure 8). Dislocations are absent, so you can be sure that any unusual peak shapes represent the true nature of the sample and are not artifacts.

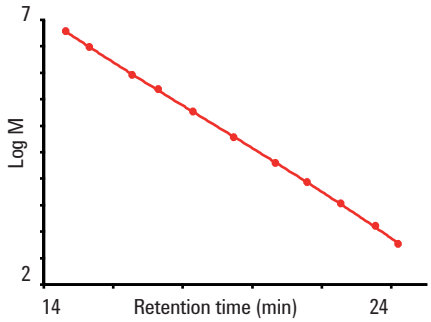


Figure 8. *Careful blending delivers highly linear polystyrene calibrations with PLgel Olexis in TCB*

The quality of the blending in PLgel Olexis columns means that polyolefins of very different polydispersity can be confidently analyzed on the same column set. Once again, PLgel Olexis provides trustworthy, clean and mono-modal peaks.

Polyolefin applications

The applications in this booklet illustrate the diversity of polyolefin samples, and reveal the flexibility of PLgel columns and the necessity for the PL-GPC 220 in addressing the analysis of such compounds.

Columns for high-molecular-weight polyolefins

Polyolefins range from low-molecular-weight hydrocarbon waxes to ultra-high-molecular-weight rigid plastics. The molecular weight distributions of polyolefins is directly related to physical properties such as toughness, melt viscosity and crystallinity. High-molecular-weight polyolefins tend to exhibit very broad molecular weight distribution (MWD). For such samples, small particles with small pore sizes are not desirable since shear degradation may occur, and so the high-pore-size particles of PLgel Olexis are recommended.

Conditions

Samples:	Polyethylenes
Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	TCB + 0.015% BHT
Flow Rate:	1 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220 (RI) + viscometer

Artifacts known as dislocations can arise in blended columns, resulting from a mismatch of the pore volume of components in the blend. Dislocations lead to false modalities and polydispersities. Avoiding dislocations was an integral part of the design brief for PLgel Olexis columns. Accurate blending of these components produces a column that gives a smooth molecular weight distribution, providing a true reflection of the shape of the MWD (Figure 9). PLgel Olexis is perfect for studies that require accurate polydispersity index and modality information.

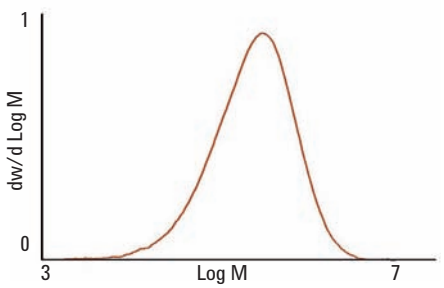


Figure 9. *True representation of polyolefin molecular weight distribution with PLgel Olexis*

Figure 10 shows a range of polyolefin samples analyzed on a PLgel Olexis column, covering the spread of molecular weights. There are no dislocations and the peak shape of the very broad samples shows true sample modality.

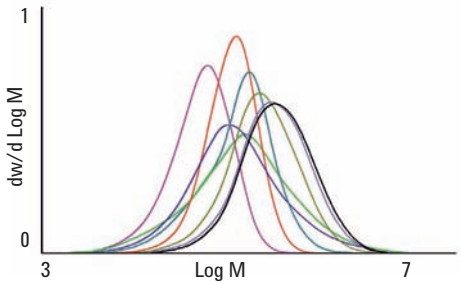


Figure 10. *PLgel Olexis reveals true modalities across the range of polyolefins*

Given the accurate resolving power of PLgel Olexis you can be sure that unusual peak shapes are real and not artifacts; unusual peak shapes of some samples will be true reflections of their modality. This is important for studies into reaction mechanisms and catalyst behavior (Figure 11).

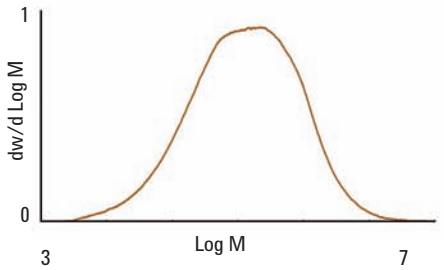


Figure 11. *A true change in peak shape revealed by PLgel Olexis of a multi-modal material manufactured from a multi-site catalyst*

Columns for lower-molecular-weight polyolefins

Crude oil, or petroleum, is the main source of organic chemicals for industry. The major chemicals are derived from two constituents of oil, xylene and naphtha. These raw materials are then broken down into more basic products, e.g. polyethylene, polypropylene, elastomers, asphalts and liquid hydrocarbons. Characterization of such products is commonly achieved using GPC. This involves a liquid chromatographic separation from which a molecular weight distribution calculation can be made following calibration of the system with suitable polymer standards. The diversity of petroleum products demands a variety of GPC column types for optimized analysis. Low-molecular-weight liquid hydrocarbons require high resolution of individual components. This is illustrated in Figure 12, where three linear hydrocarbons are resolved easily to base-line in a reasonably short analysis time.

Conditions

Samples:	Linear hydrocarbons
Columns:	2 x Agilent PLgel 5 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6520)
Eluent:	TCB
Flow Rate:	1 mL/min
Temp:	145 °C
Detector:	PL-GPC 220

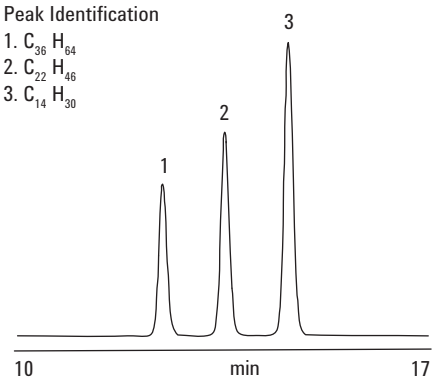


Figure 12. *Linear hydrocarbons separated to base-line on a PLgel column set*

Figure 13 shows the separation of a selection of low-molecular-weight linear hydrocarbons.

Conditions

Samples:	Linear hydrocarbons
Columns:	2 x Agilent PLgel 3 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6320)
Eluent:	TCB
Flow Rate:	0.8 mL/min
Inj Vol:	20 µL
Temp:	145 °C
Detector:	PL-GPC 220

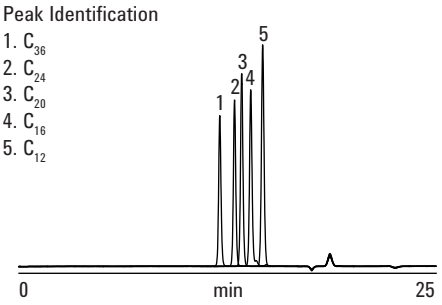


Figure 13. *Separation of low-molecular-weight hydrocarbons*

The PLgel 100Å columns have a GPC exclusion limit of 4,000 molecular weight (polystyrene equivalent). Intermediate products can be analyzed using the PLgel MIXED-D column that has a linear molecular weight resolving range up to an exclusion limit of around 400,000 molecular weight. The 5 µm particle size maintains high column efficiency and thus fewer columns are required and analysis time is relatively short.

Figure 14 shows a chromatogram of a relatively low-molecular-weight hydrocarbon wax obtained on PLgel 5 µm MIXED-D columns.

Conditions

Samples:	Linear hydrocarbons
Columns:	2 x Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent:	TCB
Flow Rate:	1 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220

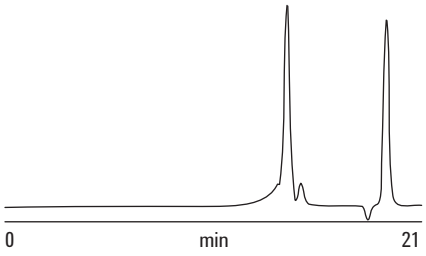


Figure 14. *A low-molecular-weight wax*

Figure 15 shows the analysis of an asphalt used in road surfacing. Subsequently derived information regarding the molecular weight distribution of such materials is invaluable in determining their processibility and final properties.

Conditions

Columns:	2 x PLgel 5 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent:	THF
Flow Rate:	1 mL/min
Temp:	40 °C
Detector:	RI

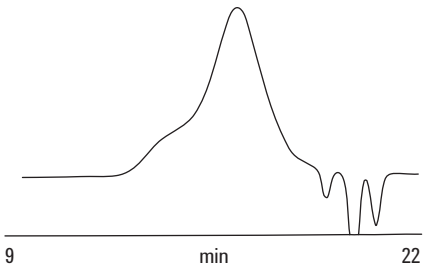


Figure 15. *Fast analysis of asphalt on PLgel 5 µm MIXED-D columns*

Repeatability study 1

A commercial sample of high-density polyethylene (HDPE) was prepared at 2 mg/mL using the PL-SP 260VS Sample Preparation System, with a dissolution temperature of 160 °C and a dissolution time of two hours. Eight aliquots of the master batch solution were dispensed into PL-GPC 220 autosampler vials and placed in the autosampler carousel of the PL-GPC 220 where the hot zone temperature was 160 °C and the warm zone 80 °C (Figure 16).

Conditions

Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent:	TCB + 0.0125% BHT
Flow Rate:	1 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220

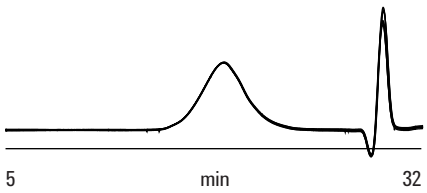


Figure 16. *Overlay of the raw data chromatograms obtained for eight consecutive injections of HDPE*

The data were analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene equivalent molecular weight averages that are shown in Table 4.

Polystyrene in TCB[1] $K = 12.1 \times 10^{-5}$ $\alpha = 0.707$

Polyethylene in TCB[2] $K = 40.6 \times 10^{-5}$ $\alpha = 0.725$

Table 4. *Summary of results from eight injections of HDPE*

Injection number	Mn	Mp	Mw
1	17,289	76,818	333,851
2	16,988	77,434	335,496
3	17,428	77,514	332,616
4	17,521	77,052	335,635
5	17,348	76,520	334,212
6	17,487	77,728	333,511
7	16,898	77,578	335,642
8	17,457	77,288	334,923
Mean	17,302	77,241	334,485
Std Dev	220	387	1,048
% Variation	1.3	0.5	0.3

Figure 17 shows an overlay of the molecular weight distribution calculated for the eight consecutive injections of the HDPE sample, and illustrates the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 µm MIXED-B columns.

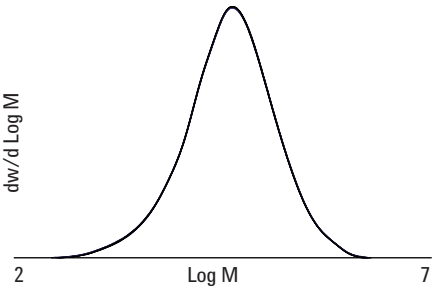


Figure 17. *Molecular weight overlay of eight consecutive injections of HDPE*

Repeatability study 2

A commercial sample of high-density polypropylene (HDPP) was prepared at 1.5 mg/mL using the PL-SP 260VS Sample Preparation System with a dissolution temperature of 160 °C and a dissolution time of two hours. Six aliquots of the master batch solution were dispensed into PL-GPC 220 autosampler vials and placed in the carousel where the hot zone temperature was 160 °C and the warm zone 80 °C.

Figure 18 shows an overlay of the raw data chromatograms obtained for six consecutive injections of the sample.

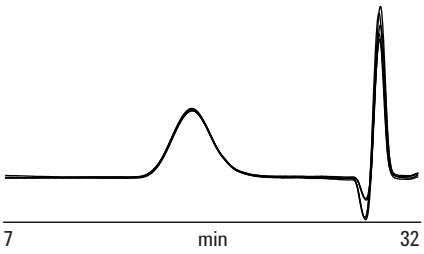


Figure 18. *Overlay of the raw data chromatograms obtained for six consecutive injections of HDPP*

The data were analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene-equivalent molecular weight averages that are shown in Table 5.

Polystyrene in TCB[1] $K = 12.1 \times 10^{-5}$ $\alpha = 0.707$

Polypropylene in TCB[2] $K = 19.0 \times 10^{-5}$ $\alpha = 0.725$

Table 5. *Overlay of the raw data chromatograms obtained for six consecutive injections of HDPP*

Injection number	Mp	Mn	Mw
1	127,132	65,086	185,795
2	131,893	65,089	185,236
3	128,673	66,802	186,202
4	132,062	67,417	188,048
5	131,625	69,320	188,679
6	130,227	69,677	186,188
Mean	130,202	67,232	186,691
Std Dev	1,693	1,815	1,239
% Variation	0.13	2.70	0.66

Conditions

Columns:	3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent:	TCB + 0.0125 BHT
Flow Rate:	1 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220

Figure 19 shows an overlay of the molecular weight distribution calculated for the six consecutive injections of the HDPP sample that illustrates the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 µm MIXED-B columns.

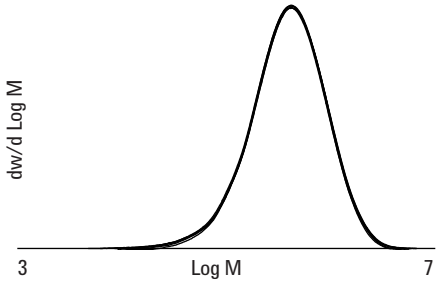


Figure 19. *Molecular weight overlay of six consecutive injections of HDPP*

References

[1] H. Coll and D. K. Gilding (1970) Universal calibration in GPC: a study of polystyrene, poly-α-methylstyrene, and polypropylene. *Journal of Polymer Science Part A-2: Polymer Physics*, 8, 89-103.

[2] T. G. Scholte , N. L. J. Meijerink, H. M. Schoffeleers and A.M.G. Brands (1984) Mark-Houwink equation and GPC calibration for linear short chain branched polyolefins, including polypropylene and ethylene-propylene copolymers. *Journal of Applied Polymer Science*, 29, 3763.

Specialist detectors

Multi-detector options for polyolefin analysis

Conventional GPC employs a refractive index or other concentration detector. However, polyolefins can be analyzed by multi-detector GPC that combines a concentration detector with a viscometer, a static light scattering detector, or both.

GPC viscometry – analysis using a concentration detector and viscometer

A viscometer may be housed inside the oven of the PL-GPC 220 to allow analysis of polyolefins by GPC viscometry. Using GPC viscometry, molecular weights are determined using the Universal Calibration method. A plot of molecular size as log (molecular weight x intrinsic viscosity) versus retention time is constructed for a series of narrow standards, based on the relationships in Equations 1 and 2.

Equation 1:

Hydrodynamic volume α molecular weight x intrinsic viscosity

Equation 2:

Log (MW x intrinsic viscosity) versus retention time ≃ log (hydrodynamic volume) versus retention time

PLgel Olexis columns are separated and calibrated in terms of size and so a Universal Calibration is obtained (Figure 20).

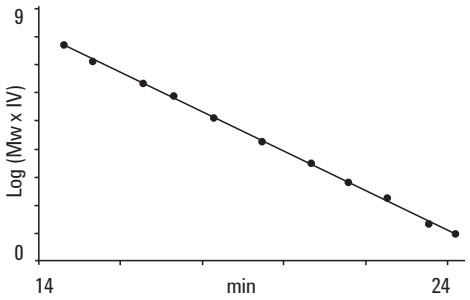


Figure 20. *Multi-detector GPC Universal Calibration of a PLgel Olexis column*

The Universal Calibration technique gives polyolefin molecular weights regardless of the calibrants used in the analysis. This allows cheaper calibrants such as polystyrene to be used while still providing accurate polyolefin results.

- Intrinsic viscosities are measured from the viscometer and concentration detector
- Accurate molecular weights are calculated assuming that the sample obeys the Universal Calibration (pure size exclusion is obtained)
- Radius of gyration is calculated using a model for the polymer behavior in solution

GPC light scattering – analysis employing a concentration detector and a light scattering detector

A dual-angle light scattering detector can be sited inside the oven of the PL-GPC 220 to allow analysis of polyolefins by GPC light scattering, employing the dissymmetry method. In GPC light scattering, accurate molecular weights are determined directly by using the response of the light scattering detector and the intensity of scattered light, as described in Equation 3.

Equation 3:

$R_\theta = CM\,(dn/dc)^2\,P_\theta K_\theta$

R_θ is the detector response, CM is concentration x mass, dn/dc is the specific refractive index increment, P_θ is the particle scattering function and K_θ is the light scattering constant.

- Molecular weights are calculated directly from the light scattering response, calculating the particle scattering function from the ratio of intensities at 15° and 90°
- Radius of gyrations are determined from the particle scattering function by comparison of the two angles, but only if the molecule is over about 10 nm in size and the scattering intensity shows angular dependence
- Intrinsic viscosity is calculated using a model for the polymer behavior in solution

GPC triple detection – analysis using concentration, viscometry and light scattering data

In this technique, both a viscometer and a dual-angle light scattering detector are housed inside the PL-GPC 220. With GPC triple detection, molecular weights are determined directly using the response of the light scattering detector as described above.

- Molecular weights are calculated directly from the light scattering response, calculating the particle scattering function from the ratio of intensities at 15 ° and 90 °
- Radius of gyrations are determined from the particle scattering function by comparison of the two angles but only if the molecule is over about 10 nm in size and the scattering intensity shows angular dependence
- Intrinsic viscosity is calculated from the viscometer trace

Comparisons between conventional GPC, GPC viscometry, GPC light scattering and GPC triple detection

Conventional GPC using only a concentration detector generates molecular weights on the basis of comparison to a series of calibration standards. However, unless the standards and samples are of the same chemistry and therefore same size in solution at any given molecular weight, the results are only relative as the GPC column separates on the basis of size not molecular weight. Conventional GPC only gives accurate results if standards of the same chemistry as the samples under investigation are used.

GPC viscometry and GPC light scattering, or GPC triple detection, can be used to determine 'absolute' molecular weights of samples, independent of the chemistry of standards used in the column calibration (GPC viscometry) or independent of column calibration entirely (GPC light scattering and GPC triple detection).

The values of molecular weight can vary between these techniques because the viscometer and light scattering detectors respond to different properties of the polymer, the viscometer to molecular density, and the light scattering detector to size in solution. Therefore, molecular weights calculated by these approaches will not necessarily have the same values.

Branching

Comparing long-chain branching in polyethylenes

Multi-detector GPC combined with branching calculations is an excellent way of comparing and identifying different kinds of polyethylene. These different materials, although of the same basic chemical structure, differ in their mode of manufacture and have very different physical properties.

LDPE – low-density polyethylene

Low-density polyethylene was the first grade of polyethylene manufactured in the 1930s. It exhibits relatively low crystallinity compared to other forms of polyethylene due to the presence of long branches on the polymer backbone (on about 2% of the carbon atoms). As a result, the tensile strength of the material is lower while resilience is higher. These long-chain branches are a result of 'backbiting' reactions in the synthetic processes used to manufacture the material. Multi-detector GPC can measure the level of branching in LDPE.

HDPE – high-density polyethylene

High-density polyethylene is manufactured using different catalysts than those used for LDPE, selected to give very low levels of branching from the backbone. HDPE therefore has higher density and crystallinity than LDPE, resulting in a tougher, more temperature-stable product. HDPE does not display long-chain branching.

LLDPE – linear low-density polyethylene

Linear low-density polyethylene is a newer material manufactured by incorporation of small quantities of alpha-olefins such as butane, hexane or octene into the polymer. LLDPE materials are more crystalline than LDPE, but are elastomeric and have a higher tensile strength and puncture resistance. Multi-detector GPC employing a viscometer and/or light scattering detector cannot be used to investigate the branching in LLDPE as changes in the density and size of the molecules compared to linear materials are very small and cannot be detected. GPC-FTIR is employed for short-chain branching analysis, as discussed on page 24.

Investigating branching in polyolefins

In multi-detector GPC, branching is assessed by investigating changes in molecular size or intrinsic viscosity as a function of increasing molecular weight. In all cases for polymers of the same chemistry, branched molecules always have lower Rg and IV values than linear analogs due to the presence of branch points.

In all methods, branching calculations can be performed on either the intrinsic viscosity (measured or calculated) or radius of gyration (measured or calculated) data. The quality of the branching results will depend on the quality of the source data (intrinsic viscosity or radius of gyration). Contraction factors are determined from the Mark-Houwink (log intrinsic viscosity versus log MW) or conformation (log radius of gyration versus log MW) plots using the relationships in Equation 4.

Equation 4:

Radius of gyration contraction factor

$$g = \left(\frac{\text{Rg branched}}{\text{Rg linear}} \right)_{MW}$$

Intrinsic viscosity contraction factor

$$g' = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)_{MW}$$

where $g = g'^{(1/\epsilon)}$

ϵ (structure factor) = 0.5 to 1.5, typically 0.75

The value of g (directly or taken from the value of g' and an estimation of the structure factor, typically 0.75) is used along with the branching repeat unit (the molecular weight of the monomer multiplied by 1,000) to obtain branching numbers using a branching model. In the absence of structural data for the sample, a number-average ternary-branching model is used as shown in Equation 5.

Equation 5:

$$g = [(1 + B_n/7)^{1/2} + 4B_n/9\pi]^{-1/2}$$

where B_n = branches per 1,000 carbons

Branching numbers are expressed as number of branches per 1,000 carbons (from polyethylene investigations). If the polymer in question is not polyethylene then the actual branching number may not be directly meaningful. However, comparison between samples is still possible.

Analysis of branching in polyethylenes

Samples of LDPE, HDPE and LLDPE were analyzed with the PL-GPC 220 by triple detection.

Conditions

Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	TCB + 0.015% BHT
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220 (RI) + viscometer + dual-angle light scattering

Refractive index, dual-angle light scattering and viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software. A polystyrene standard was used to generate the detector constants for the triple detection analysis.

Figure 21 shows the molecular weight distributions for the three samples. Although there was some overlap, the samples clearly had significantly different molecular weights.

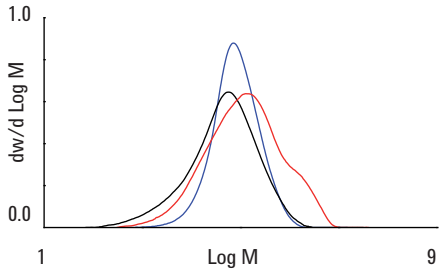


Figure 21. *Overlaid molecular weight distributions for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red*

Figure 22 shows the Mark-Houwink plots for the three samples using intrinsic viscosities generated from the viscometer and molecular weights from the light scattering detector.

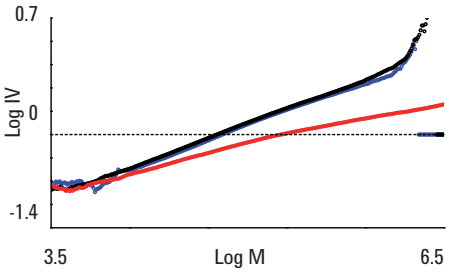


Figure 22. *Overlaid Mark-Houwink plots for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red*

The Mark-Houwink plot describes the change in the viscosity of the polymers as a function of increasing molecular weight. The HDPE and LLDPE samples overlay on the Mark-Houwink plot, indicating that the polymers have very similar structures. The Mark-Houwink parameters K (the intercept) and alpha (the slope) indicate that the materials contain no branching that can be detected by multi-detector GPC. However, the LDPE shows a clear deviation from the HDPE and LLDPE lines, with a decreasing slope as molecular weight increases. This is due to increased branching of the LDPE compared to the other materials as molecular weight increases lead to a reduction in viscosity.

Branching analysis of polyethylenes with Cirrus GPC Multi Detector Software

The presence of long-chain branching (over six carbons in length) in polyolefins strongly influences physical properties such as melt viscosity and mechanical strength. The distribution chain branches in polyolefins are determined by the polymerization mechanism and there is significant interest in the production of materials with well-defined and characterized molecular weight and branching distributions for specific applications.

Three samples of polyethylene, one HDPE and two LDPE, were analyzed using the PL-GPC 220 by GPC/viscometry. Two of the samples had been synthesized by a mechanism to promote branching, while the third was a standard linear reference material, NBS 1475.

Refractive index viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software using the Universal Calibration approach. Polystyrene standards were used to generate the Universal Calibration and the unbranched sample was used as a linear model in the determination of branching.

Figure 23 shows the molecular weight distributions for the three samples. The black plot is for the unbranched sample. Although there was some overlap, the samples clearly had significantly different molecular weights.

Figure 24 shows the Mark-Houwink plots for the three samples. The upper-most sample is the unbranched material. The other two samples have lower intrinsic viscosities at any given molecular weight, with the unbranched polymer indicating the presence of branching. This can be expressed in terms of g, the branching ratio, defined in Equation 6, where ϵ is a constant.

Equation 6:

$$g = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)^{1/\epsilon}$$

Conditions

Samples:	Polyethylenes
Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	TCB + 0.015% BHT
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220 (RI) + viscometer

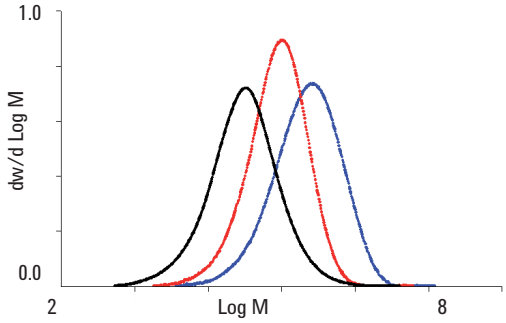


Figure 23. *Molecular weight distribution plots for three polyethylene samples – the black plot is the unbranched sample*

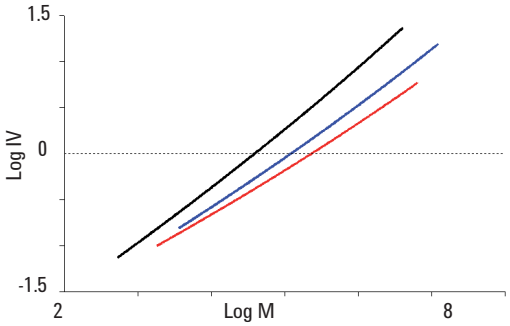


Figure 24. *Mark-Houwink plots for three samples of polyethylene*

The unbranched sample was used as the linear model and so gives a g value of unity (except at high molecular weight due to scatter in the data). The other two samples both exhibit a decrease in g as a function of molecular weight, indicating that as molecular weight increases the number of branches also increases. Based on these calculated g values, a branching number or number of branches per 1,000 carbon atoms can be generated. This is achieved by fitting the data into a model. The Cirrus GPC Multi Detector Software offers a selection of branching models that can be employed in this approach. In this case a model was used that calculates a number-average branching number assuming a random distribution of branches on the polymer. Figures 25 and 26 show the g plots and branching number plots obtained for the samples.

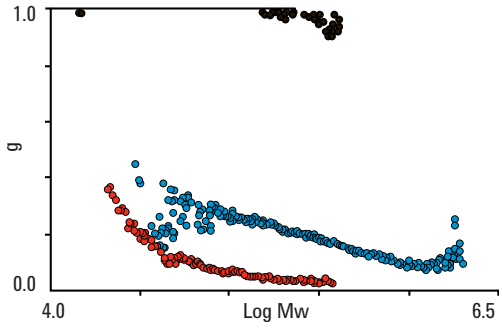


Figure 25. *Branching ratio g plots for three polyethylene samples – the black plot is the unbranched sample*

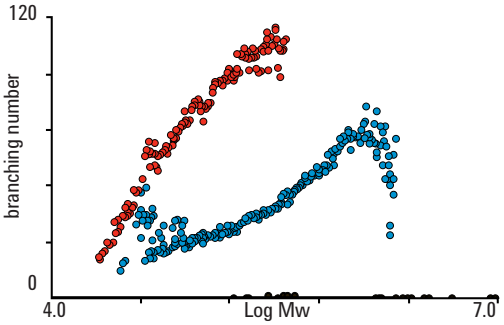


Figure 26. *Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample*

The results show that of the two, branched samples, the trend in molecular weight distribution does not follow the trend in branching distribution. The sample showing the most branching at any given molecular weight has a lower molecular weight than the second sample. Clearly, understanding both the molecular weight and branching distributions will give an insight into the processibility of the two materials.

Analysis of branching in linear low-density polyethylene (LLDPE)

Fourier transform-infrared (FTIR) spectroscopy is a well-established technique used in compositional analysis of materials through the measurement of vibrational absorption bands. Polymers typically exhibit relatively simple absorption spectra, allowing them to be readily identified by comparison to library data and are therefore well suited to analysis by FTIR. Coupling FTIR detection with gel permeation chromatography is particularly advantageous as FTIR detection can be utilized as both concentration detector for molecular weight calculations and as a spectroscopic tool for compositional analysis, significantly enhancing the information available from a single GPC experiment.

Coupling a PL-GPC 220 system to one of the range of Agilent's FTIR spectrometers can be achieved using the PL-HTGPC-FTIR interface, which consists of a heated flow cell, a heated transfer line, and a temperature control box. The flow cell and transfer line can be heated up to 175 °C with an accuracy of ± 0.5 °C for polyolefin applications. To obtain good quality spectra, the FTIR spectrometer is fitted with a fast MCT (mercury-cadmium-telluride) detector. Data acquisition is performed through the spectrometer's time-resolved data-acquisition software.

GPC/FTIR analysis of polyethylene

Highly crystalline polyethylene is difficult to analyze by GPC due to its limited solubility in most organic solvents, and the high temperatures required for dissolution (typically over 135 °C). Trichlorobenzene (TCB) is the most commonly used solvent for these materials. TCB is also a suitable solvent for GPC analysis with FTIR detection as the solvent has a good absorption window between about 3,500 and 2,700 cm^{-1}, which corresponds to the >C-H stretching region. CH vibrations dominate the solid-state spectra of polyethylene and so this absorption region is of key importance.

Focusing on the >C-H stretching region, differences in the proportions of $>CH_2$ and $–CH_3$ groups in a sample can be seen in the relative intensities of the absorption bands. This dependence of the infrared spectra on the presence of $–CH_3$ and $>CH_2$ groups can be used to measure the level of short-chain branching (SCB) in polyethylene[1]. These are branches less than six carbons long introduced by co-polymerization of ethylene with other alpha-olefins that cannot be detected by traditional multi-detector GPC experiments, as they do not affect the viscosity of the polymer. The level of SCB does, however, strongly influence crystallinity, density, and stress-crack resistance of polyethylene. By measuring the spectra of polyethylene containing SCB, the relative intensities of the stretching vibrations due to $–CH_3$ and $>CH_2$ groups can be measured and, providing that the monomers used to introduce SCB are known, the level of SCB can be estimated using chemometrics. Coupling the detector to a GPC system allows the SCB to be assessed (as a function of molecular weight).

Analysis of an ethylene-hexene copolymer by GPC/FTIR

A sample of ethylene co-polymerized with hexane was analyzed using the PL-GPC 220 coupled to an Agilent FTIR to assess the levels of short-chain branching.

Conditions

Column:	2 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	Trichlorobenzene (with BHT)
Inj Vol:	200 µL
Flow Rate:	1.0 mL/min
Temp:	160 °C
Data Collection:	Time-resolved Agilent Resolutions Pro software collecting at 8.0 cm^{-1} resolution with 16 scan accumulations for 11 minutes, range 3,500 – 2,700 cm^{-1} with automatic solvent background subtraction
Detection:	Agilent PL-HTGPC-FTIR interfaced to an Agilent FTIR spectrometer fitted with an MCT detector

Cirrus GPC-FTIR SCB software was used to perform the experiments, calculating SCB based on a rigorous chemometrics approach. To determine molecular weight, the FTIR data was used as a concentration source for the generation of Figure 27, showing an overlay of the polymer weight and short-chain branching distribution obtained for a copolymer of ethylene and another alpha-olefin by FTIR. Clearly, in this case the level of co-monomer incorporation was uniform across the distribution.

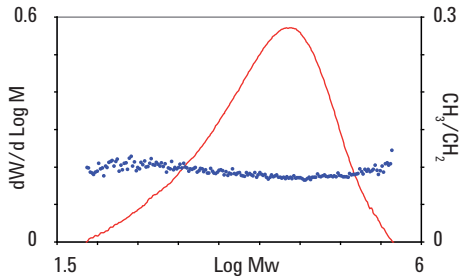


Figure 27. *Overlaid chromatogram of polymer weight and short-chain branching distribution for a sample of ethylene-hexene copolymer*

Reference

[1] P.J. DesLauriers, D.C. Rohlfing and E.T. Shieh (2002) Quantifying short chain branching microstructures in ethylene-1-olephin copolymers using size exclusion chromatography and Fourier transform infrared spectroscopy (SEC-FTIR). *Polymer*, 43, 159-170.

Ordering Information

Columns

Description	Part No.
Agilent PLgel 3 µm 100Å, 300 x 7.5 mm	PL1110-6320
Agilent PLgel 5 µm 100Å, 300 x 7.5 mm	PL1110-6520
Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm	PL1110-6504
Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm	PL1110-6100
PLgel 10 µm MIXED-B LS, 300 x 7.5 mm	PL1110-6100LS*
PLgel 20 µm MIXED-A, 300 x 7.5 mm	PL1110-6200
PLgel 20 µm MIXED-A LS, 300 x 7.5 mm	PL1110-6200LS*
Agilent PLgel Olexis, 300 x 7.5 mm	PL1110-6400

Standards

Description	Part No.
Agilent PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0301
Agilent E-M-10 polyethylene calibration kit, 10 x 0.2 g	PL2650-0101
Agilent E-MW-10 polyethylene calibration kit, 10 x 0.1 g	PL2650-0102
Agilent E-SCB polyethylene short-chain branching calibration kit, 10 x 0.1 g	PL2650-0103

Instruments

Description	Part No.
Agilent PL-SP 260VS Sample Preparation System**	
Agilent PL-GPC 220 Integrated GPC/SEC System	PL0820-0000
Agilent PL-HTGPC-FTIR**	
Agilent PL-BV 400HT Online Integrated Viscometer	PL0810-3050
Agilent PL-HTLS 15/90 Light Scattering Detector	PL0640-1200
Agilent custom accessory kit**	

Software

Description	Part No.
Agilent Cirrus GPC Multi Detector Software	PL0570-2020
Agilent Cirrus GPC Software	PL0570-2000
Agilent GPC-FTIR SCB Software	PL0570-2300

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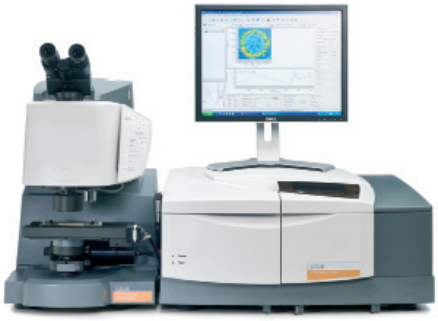

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Agilent Technologies

Analysis of engineering polymers by GPC/SEC

Application Compendium

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The Measure of Confidence

Agilent Technologies

Contents Page

Polymer analysis by GPC/SEC

Introduction

Increasingly, plastics are being utilized to perform structural and mechanical roles in the construction and engineering industries. The favorable properties of polymers, such as mechanical strength, durability, and resistance to chemical and physical degradation, coupled with their relative cheapness, means that polymers outperform many traditional materials, including wood and metals, in key applications. With the creation of new polymeric materials, this shift towards plastics is becoming even more pronounced as materials with new properties are designed and developed. An understanding of the behavior of polymers is key to designing new materials with appropriate performance characteristics for specific applications. Analysis of these materials is therefore a critical component of the development and manufacture of engineering polymers.

Gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many of their physical properties. Generally, increasing molecular weight leads to higher performance characteristics, while an increase in the width of the distribution (the polydispersity) leads to a loss of performance but an increase in the ease of processing.

Engineering polymers are particularly difficult to analyze – they are generally tough and difficult to dissolve, often requiring aggressive solvents and elevated temperatures. For these applications at high temperature, a high performance integrated GPC system, such as the Agilent PL-GPC 220 Integrated GPC/SEC System, is a necessity. The PL-GPC 220 has the highest temperature range of any system on the market. The following applications show the analysis of various types of engineering polymers and illustrate the conditions and equipment required.

Table 1. *Effects of molecular weight distribution on the properties of engineering polymers*

	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	–
Decreasing distribution	+	+	–	+	+	+

Agilent's GPC/SEC technology

Agilent produces the most extensive range of GPC columns, standards, instruments and software, ideally suited to the analysis of engineering polymers.

GPC/SEC columns

Agilent's GPC columns are the most rugged and reliable on the market, making them ideal for applications performed in viscous solvents and at elevated temperatures where column lifetime can be an issue. The extensive column range includes products tailored to the analysis of engineering polymers that generally have high molecular weights and high viscosities, and includes specialist columns such as Agilent PLgel Olexis that are designed for the analysis of a specific material. With extensive options in particle and pore size, Agilent's columns can be selected to match the molecular weight of the material under investigation, thereby ensuring that the best quality of data is obtained from the GPC/SEC experiment.

Agilent offers a selection of GPC/SEC column dimensions

GPC/SEC standards

Narrow polydispersity polymer standards with very highly characterized molecular weights are used as calibration standards in the GPC analysis of engineering polymers. Polystyrene standards are the first choice for many organic solvents, either for conventional GPC column calibration or for calibrating light scattering and viscosity detectors.

Agilent's EasiVial calibration kit

GPC/SEC instruments

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

Agilent's PL-GPC 220 Integrated GPC/SEC System features unbeatable reproducibility for any GPC/SEC application, across the entire operating range. The PL-GPC 220 is an extremely flexible system, designed to run almost all polymer, solvent and temperature combinations, from 30 to 220 °C.

The PL-GPC 220 allows all forms of the GPC/SEC experiment to be performed and can be used to analyze the complete range of engineering materials, including those that require analysis at extremely high temperatures. Multiple detection options can be included in the instruments, such as light scattering and viscometry, and dedicated analysis software is available that allows the properties of engineering polymers to be analyzed in detail. Agilent's complete range of columns and instrumentation offer a clear advantage in the analysis of engineering polymers.

PL-GPC 220 Integrated GPC/SEC System

For more information on Agilent's GPC/SEC products, visit **www.agilent.com/chem/gpcsec**

GPC/SEC of polymers in aggressive solvents

Aggressive solvents

Many polymers, especially those used in engineering applications, show only limited solubility in a small number of solvents. This is because high strength and toughness are usually a result of high molecular weight and/or high crystallinity. Increasing molecular weight requires untangling the molecular chains to dissolve the material, whereas increased crystallinity requires break-up of any inter-chain bonds that may be present.

The PL-GPC 220 Integrated GPC/SEC System is designed to allow the use of even the most aggressive solvents. The following applications illustrate the analysis of a range of engineering polymers that require aggressive solvents for solubility or as eluents during the analysis.

GPC analysis of polyether ether ketone (PEEK)

Application areas: High performance components, tubing in liquid chromatography

Polyether ether ketone (PEEK) was developed in 1977 by ICI and was one of the first of the new generation of engineering thermoplastics developed for chemical resistance, high mechanical strength and high thermal stability – the useful properties of the material are retained up to temperatures as high as 315 °C. A crystalline material with repeat units of two ethers and a ketone group in the polymer backbone, PEEK is a high cost material. For many applications, such as the manufacture of piston components in engines, the insulation of cables and the production of high performance aircraft parts, this cost is justified as there are no other plastics that can offer the same performance properties. The industrial performance of PEEK makes analysis of this material by GPC difficult. PEEK has excellent chemical resistance and is unaffected by many organic and inorganic chemicals, dissolving only in strong or concentrated anhydrous oxidizing agents. Previous methods for analyzing PEEK have involved mixtures of trichlorobenzene and phenol running at high temperatures.

For this analysis, the PEEK sample was dissolved in a small volume of dichloroacetic acid at 120 °C for two hours. After dissolution, the sample was diluted to the required concentration of 0.2% (w/v) with chloroform and injected into a system running at temperature after filtration to remove undissolved material.

The PEEK sample eluted as a broad polymer peak with an MW of 70,000 g/mol and a polydispersity of 2.2. The large system peak observed at the end of the run was due to the excess dichloroacetic acid used in the preparation of the sample.

Conditions

Sample:	Polyether ether ketone (PEEK)
Columns:	2 x Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent:	80% chloroform 20% dichloroacetic acid
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Detector:	PL-GPC 220

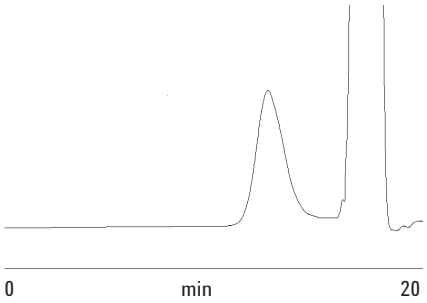

Figure 1. *Chromatogram of a PEEK sample*

GPC analysis of polybutylene terephthalate (PBT) resins in HFIP

Application areas: Machined parts

Polybutylene terephthalate (PBT) resins are used in a wide variety of applications in which toughness and resistance to damage are highly advantageous. However, mechanical and thermal stress during the production of molded parts can cause degradation, giving a reduction in desirable physical properties. The molecular weight distribution of the resin is a key measure of the onset of degradation and therefore of estimating the mechanical strength of the final product. PBT is soluble in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), a polar organic solvent, which is excellent for dissolving polar polymers such as polyamides and polyesters. The analysis was carried out in HFIP modified by the addition of 20 mM sodium trifluoroacetate to prevent aggregation. Two Agilent PL HFIPgel columns, designed specifically for HFIP applications, were employed for the analysis at a temperature of 40 °C. The PL-GPC 220 Integrated GPC/SEC System was used with differential refractive index and viscometry detection. GPC coupled with a molecular weight-sensitive viscometer allowed calculation of molecular weights based on hydrodynamic volume using the Universal Calibration approach, leading to molecular weights independent of the standards used to generate the column calibration. Agilent polymethylmethacrylate (PMMA) standards were employed to generate the Universal Calibration.

Table 2 shows the molecular weight averages and intrinsic viscosity for the sample before and after molding, as determined by GPC/viscometry. Clearly, the molecular weight distribution indicates that after molding, the material has suffered from degradation and is less robust than the virgin material.

Table 2. *Molecular weight averages and intrinsic viscosity for the PBT resin sample*

	Mn/g mol^{-1}	Mw/g mol^{-1}	Intrinsic viscosity/g^{-1}
Virgin resin	24,400	48,600	0.535
Molded part	11,200	24,000	0.306

Conditions

Samples: PBT resin
Columns: 2 x PL HFIPgel, 300 x 7.5 mm (Part No. PL1114-6900HFIP)
Eluent: HFIP + 20 mM NaTFA
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Temp: 40 °C
Detectors: PL-GPC 220, viscometer

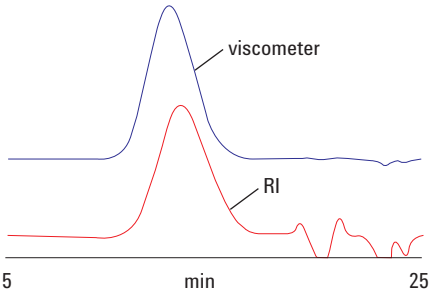

Figure 2. *Example overlay of a dual-detector chromatogram of the virgin PBT resin before molding*

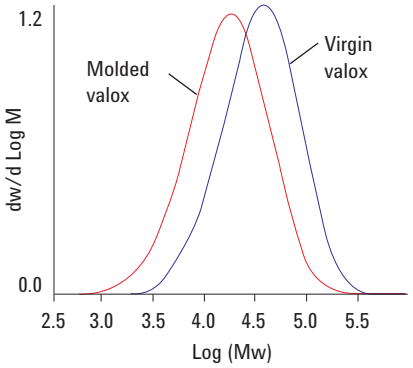

Figure 3. *Molecular weight distributions of the two samples*

Polyethylene terephthalate analysis in o-chlorophenol as an alternative solvent

As an alternative to the use of HFIP, PET can be analyzed in o-chlorophenol. This viscous solvent requires elevated temperatures and is a hazardous substance.

The samples were dissolved by heating to 110 °C for 30 minutes. The polymer remains in solution at room temperature but the high viscosity of the eluent means that high temperature GPC is necessary. Three grades of PET, with different intrinsic viscosities, were analyzed and compared, showing minor differences between the materials.

Conditions

Samples:	PET resin
Columns:	2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent:	o-chlorophenol
Flow Rate:	1.0 mL/min
Temp:	100 °C
Detection:	PL-GPC 220

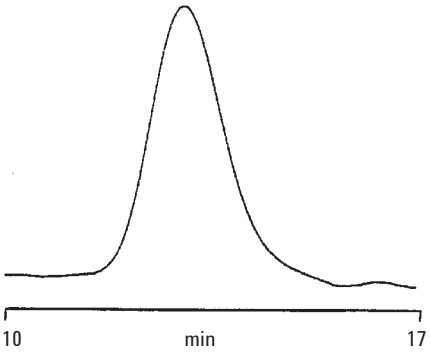

Figure 4. *Chromatogram of a PET sample*

Peak Identification
1. IV=0.72
2. IV=0.75
3. IV=0.84

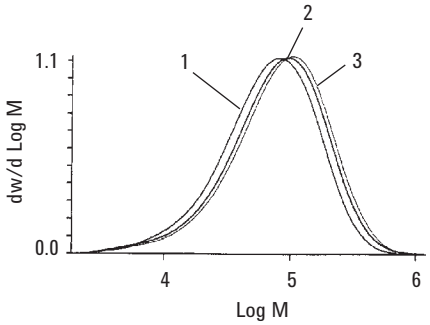

Figure 5. *Molecular weight distributions of the PET samples*

GPC/SEC of polymers at high temperature

High temperature analysis

Some highly crystalline polymers such as polyethylene show only limited solubility and only then at high temperatures. This is due to the fact that temperature is required to break down the ordered crystalline structure, and on cooling, the material will re-crystallize and precipitate from solution. For these applications, high temperature is required throughout the entire analysis to ensure that the samples remain in solution during the experiment. The PL-GPC 220 Integrated GPC/SEC System is capable of maintaining a constant temperature up to 200 °C between the point of injection, the columns and the detector cells, until the point of elution. The following applications illustrate the analysis of crystalline polymers at high temperatures using the PL-GPC 220.

Column selection for polyolefin analysis

Polyolefins range from low molecular weight hydrocarbon waxes to ultra high molecular weight rigid plastics. The molecular weight distribution of polyolefins is directly related to physical properties such as toughness, melt viscosity, and crystallinity. GPC/SEC is widely accepted as the preferred technique to fully characterize the molecular weight distribution of polyolefins.

The selection of a column set for the analysis of a polyolefin is dependent on the molecular weight range of the sample. Low molecular weight samples can be analyzed using high efficiency, relatively low pore size columns. Higher molecular weight materials require large particle size media to minimize shear effects, with a wide pore size distribution.

Figures 6 to 8 show typical data for four very different polyolefin samples, all obtained with the PL-GPC 220.

Conditions

Sample:	Linear hydrocarbons
Columns:	2 x Agilent PLgel 3 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6320)
Eluent:	TCB
Flow Rate:	0.8 mL/min
Inj Vol:	20 µL
Temp:	145 °C
Detector:	PL-GPC 220

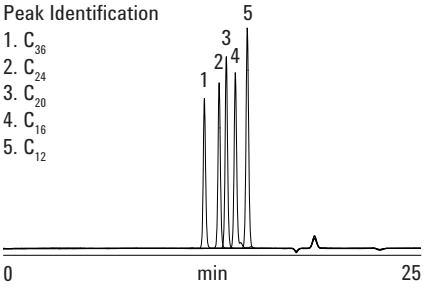

Figure 6. *Separation of a selection of low molecular weight linear hydrocarbons analyzed using two PLgel 3 µm 100Å columns*

Conditions

Sample:	Hydrocarbon wax
Columns:	2 x Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent:	TCB
Flow Rate:	1.0 mL/min
Inj Vol:	100 µL
Temp:	160 °C
Detector:	PL-GPC 220

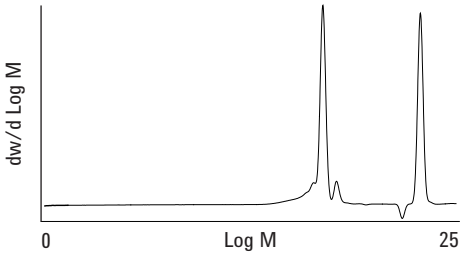

Figure 7. *Chromatogram of a relatively low molecular weight hydrocarbon wax obtained on two PLgel 5 µm MIXED-D columns*

Conditions

Sample:	Polyethylene
Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	TCB
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220

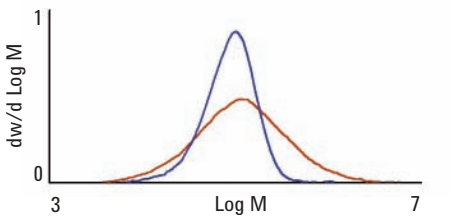

Figure 8. *Overlaid molecular weight distributions of medium and high polydispersity polyethylene samples*

High molecular weight materials require analysis on high pore size PLgel Olexis columns that minimize shear degradation, and are able to resolve up to 100,000,000 g/mol with a 13 µm particle size. These applications illustrate the diversity of polyolefin samples and indicate the flexibility of the PLgel series of columns in addressing the analysis of such samples.

High temperature GPC analysis of polypropylene on the PL-GPC 220 – repeatability study

Application areas: Plastic pipes, bottles and containers

The PL-GPC 220 Integrated GPC/SEC System is ideally suited to the analysis of polypropylene. A commercial sample of polypropylene (PP) was prepared at 1.5 mg/mL using the Agilent PL-SP 260VS Sample Preparation System with a dissolution temperature of 160 °C and a dissolution time of two hours. Six aliquots of the master batch solution were dispensed into the PL-GPC 220 autosampler vials and placed in the carousel where the hot zone temperature was 160 °C and the warm zone 80 °C.

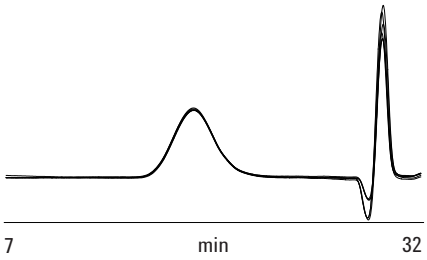

Figure 9. *Overlay of the raw data chromatograms obtained for six consecutive injections of polypropylene*

The data was analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene equivalent molecular weight averages that are shown in Table 3.

Polystyrene in TCB[1] $K=12.1x10^{-5}$ $\alpha=0.707$

Polypropylene in TCB[2] $K=19.0x10^{-5}$ $\alpha=0.725$

Table 3. *Calculated molecular weights for six injections of polypropylene and calculated % variation*

Injection Number	Mp	Mn	Mw
1	127,132	65,086	185,795
2	131,893	65,089	185,236
3	128,673	66,802	186,202
4	132,062	67,417	188,048
5	131,625	69,320	188,679
6	130,227	69,677	186,188
Mean	130,202	67,232	186,691
Std Dev	1,693	1,815	1,239
% Variation	0.13	2.70	0.66

The results illustrate the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 µm MIXED-B columns.

Conditions

Sample:	Polypropylene
Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No.PL1110-6400)
Eluent:	TCB + 0.0125% BHT
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220

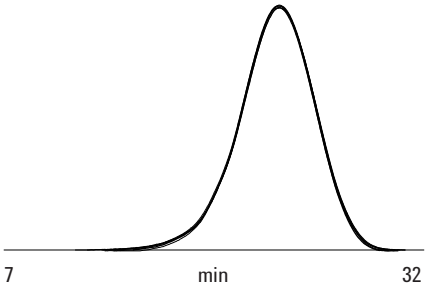

Figure 10. *Overlay of the molecular weight distribution calculated for six consecutive injections of the polypropylene sample*

References

[1] H. Col and D. K. Giddings, *J. Polym. Sci.*, (A2) **8** (1970) 89

[2] T.G. Scholte et al, *J. Appl. Polym. Sci.*, **29** (1984) 3763

Branching analysis of polyethylenes with Cirrus GPC Multi Detector Software

Application areas: Plastic bags and containers

The presence of long chain branching (over 6 carbons in length) in polyolefins strongly influences physical properties such as melt viscosity and mechanical strength. The distribution chain branches in polyolefins are determined by the polymerization mechanism and there is significant interest in the production of materials with well-defined and characterized molecular weight and branching distributions for specific applications.

Here we describe the analysis of three samples of polyethylene with the PL-GPC 220 by GPC/viscometry. Two of the samples had been synthesized by a mechanism to promote branching while the third was a standard linear reference material NBS 1475. The analysis was carried out at 160 °C with three PLgel Olexis columns in trichlorobenzene (TCB) with 0.015% butylated hydroxytoluene (BHT) as a stabilizer.

Refractive index and viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software using the Universal Calibration approach. Polystyrene standards were used to generate the Universal Calibration and the unbranched sample was used as a linear model in the determination of branching.

Figure 11 shows the molecular weight distributions for the three samples. The black plot is for the unbranched sample. Although there was some overlap, the samples clearly had significantly different molecular weights.

Figure 12 shows the Mark-Houwink plots for the three samples. The uppermost sample is the unbranched material. The other two samples have lower intrinsic viscosities at any given molecular weight, with the unbranched polymer indicating the presence of branching. This can be expressed in terms of g, the branching ratio, defined as follows, where ϵ is a constant:

$$g = \left[\frac{\text{Intrinsic viscosity (branched)}}{\text{Intrinsic viscosity (linear)}} \right]^{1/\epsilon}$$

Conditions

Samples:	Polyethylenes
Columns:	3 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	TCB + 0.015% BHT
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Temp:	160 °C
Detector:	PL-GPC 220 + viscometer

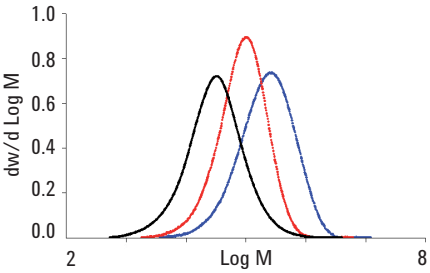

Figure 11. *Molecular weight distribution plots for the three polyethylene samples – the black plot is for the unbranched sample*

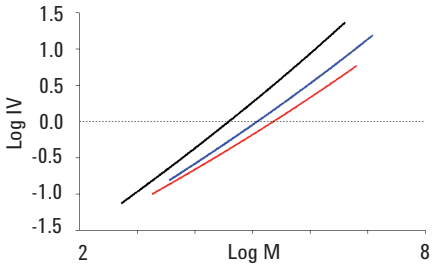

Figure 12. *Mark-Houwink plots for three samples of polyethylene*

The unbranched sample was used as the linear model and so gives a g value of unity (except at high molecular weight due to scatter in the data). The other two samples both exhibit a decrease in g as a function of molecular weight, indicating that as molecular weight increases, the number of branches increases. Based on these calculated g values, a branching number or number of branches per 1,000 carbon atoms can be generated. This is achieved by fitting the data into a model. The Cirrus GPC Multi Detector Software offers a selection of branching models that can be employed in this approach. In this case a model was used that calculates a number-average branching number, assuming a random distribution of branches on the polymer. Figures 13 and 14 show the g plots and branching-number plots obtained for the samples.

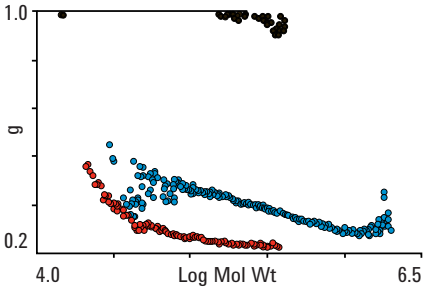

Figure 13. *Branching ratio g plots for the three polyethylene samples – the black plot is the unbranched sample*

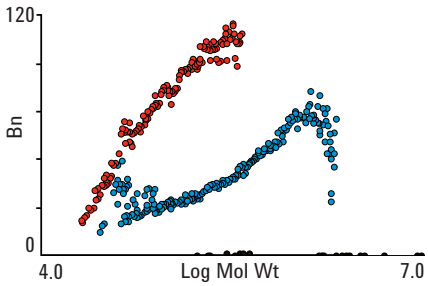

Figure 14. *Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample*

The results show that of the two branched samples, the trend in molecular weight distribution does not follow the trend in branching distribution. The sample showing the most branching at any given molecular weight has a lower molecular weight than the second sample. Clearly, understanding both the molecular weight and branching distributions will give an insight into the processibility of the two materials.

Polyphenylene sulfide analysis

Application areas: High performance membranes, felts and insulators

Polyphenylene sulfide (PPS) is an engineering polymer with a rigid backbone of alternating aromatic rings linked by sulfur atoms. It is useful as a structural material due to its high resistance to both chemical and thermal attack, and the material is very stiff, even at high temperatures. PPS is used in a number of applications, including as a filter fabric for coal boilers, in felts used in paper making, in electrical insulation applications and in the manufacture of specialty membranes. PPS is naturally insulating, although the addition of a dopant can be used to make the material semi-conducting.

PPS is particularly difficult to analyze by GPC. The high chemical and thermal resistance of the material means that it is only soluble in specialist solvents such as ortho-chloronaphthalene at elevated temperatures of around 200 °C. The PL-GPC 220 is capable of operation at these temperatures, and the PLgel column material can perform the analysis of PPS.

Conditions

Columns: 3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent: o-chloronaphthalene
Flow Rate: 1.0 mL/min
Temp: 210 °C
Detector: PL-GPC 220

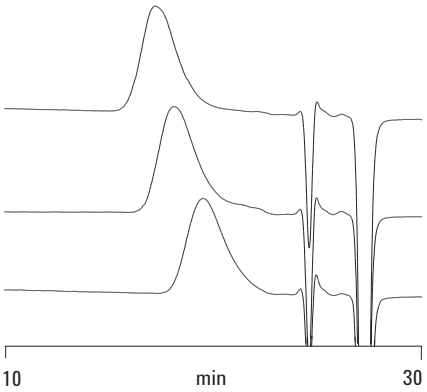

Figure 15. *Overlaid chromatograms of three samples of polyphenylene sulfide*

Ordering information

The following products are featured in this application compendium. For a full list of GPC/SEC part numbers, visit **www.agilent.com/chem/store**

Columns

Description	Part No.
Agilent PLgel 3 µm 100Å, 300 x 7.5 mm	PL1110-6320
Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm	PL1110-6504
Agilent PLgel 5 µm MIXED-C, 300 x 7.5 mm	PL1110-6500
Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm	PL1110-6100
Agilent PLgel 20 µm MIXED-A, 300 x 7.5 mm	PL1110-6200
Agilent PLgel 20 µm MIXED-A LS, 300 x 7.5 mm	PL1110-6200LS*
Agilent PLgel Olexis, 300 x 7.5 mm	PL1110-6400
Agilent PL HFIPgel, 300 x 7.5 mm	PL1114-6900HFIP

Standards

Description	Part No.
Agilent PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0301
Agilent PS-L EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0401
Agilent EasiCal PS-1 pre-prepared polystyrene kit	PL2010-0501
Agilent EasiCal PS-2 pre-prepared polystyrene kit	PL2010-0601
Agilent PM EasiVial 2 mL pre-weighed polymethylmethacrylate calibration kit	PL2020-0201
Agilent PM EasiVial 4 mL pre-weighed polymethylmethacrylate calibration kit	PL2020-0200

Instruments

Description	Part No.
Agilent PL-GPC 220 Integrated GPC/SEC System	PL0820-0000
Agilent PL-BV 400HT Online Integrated Viscometer	PL0810-3050
Agilent PL-HTLS 15/90 Light Scattering Detector	PL0640-1200
Agilent PL-SP 260VS Sample Preparation System**	

Software

Description	Part No.
Agilent Cirrus GPC Software	PL0570-2000
Agilent Cirrus GPC Multi Detector Software	PL0570-2020

** Low shedding for light scattering applications*
*** Contact your local sales office or distributor for different options*

Companion products

The term engineering polymers includes a wide range of materials, and although gel permeation chromatography is the paramount technique in their analysis, there are other analytical techniques that may be employed. Agilent makes a range of instruments in molecular spectroscopy and X-ray crystallography that can be used for the investigation of these types of material. Agilent's advanced instruments elucidate their characteristics and composition, leading to a better understanding of the behavior and fitness for purpose of these increasingly valuable products.

UV-Vis-NIR spectroscopy

Agilent's Cary range of UV-Vis-NIR spectrophotometers has been synonymous with excellence and high performance for over 60 years. The Cary spectrophotometer series is the standard for researchers wanting to extend the boundaries of spectrophotometric measurement. The range is equally at home in routine laboratories where reliability and ease of use are vital for the quality control of polymers.

The Agilent 600-IR series provides the highest level of sensitivity combined with detailed structural and compositional information for information-rich detection

Fluorescence spectroscopy

Agilent's Cary Eclipse Fluorescence Spectrophotometer offers the high performance you've come to expect from a Cary, at a surprisingly low price. With xenon flash lamp technology, plug-and-identify electronics and feature-packed, intuitive software, the instrument embodies the Agilent and Cary names.

FTIR spectroscopy

The compositional analysis of polymers is made easy with Agilent's FTIR spectrometers and microscopes that provide the ability to extract specific chemical information from extremely small sample areas. Exclusive to Agilent and taking full advantage of our FTIR attenuated total reflection (ATR) imaging technology, the Specac Imaging Golden Gate Diamond ATR accessory also provides the highest quality chemical images that are distortion- and aberration-free with a preserved aspect ratio, while maintaining the Golden Gate's robustness and ease of use.

Raman spectroscopy

Raman spectroscopy delivers qualitative and quantitative information on chemical species that make up engineering polymers. Raman complements IR spectroscopy, particularly for the study of crystalline polymers. Agilent's Synergy FT-Raman module is the most compact FT-Raman accessory on the market, maintaining the versatility required by research spectroscopists.

X-ray crystallography

X-ray crystallography was famously used to decipher the structure of the DNA polymer in the early 1950s. These days, Rosalind Franklin would probably use Agilent's Xcalibur E, the expert diffractometer for the modern chemical crystallography laboratory and the most popular choice for single wavelength, small molecule crystallography.

Other GPC/SEC resources from Agilent

Agilent has published application compendia on biodegradable polymers, polyolefin analysis, elastomers, 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
Biodegradable polymers	5990-6920EN
Polyolefin analysis	5990-6971EN
Elastomers	5990-6866EN
Low molecular weight resins	5990-6845EN
Introduction to GPC/SEC	5990-6969EN
GPC/SEC reference poster	5990-6882EN
Column selection guide	5990-6868EN

Search for all our publications in the Literature Library at **www.agilent.com/chem/library**

Notes

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Agilent Technologies

Biodegradable polymers - analysis of biodegradable polymers by GPC/SEC

Application compendium

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The Measure of Confidence

Agilent Technologies

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

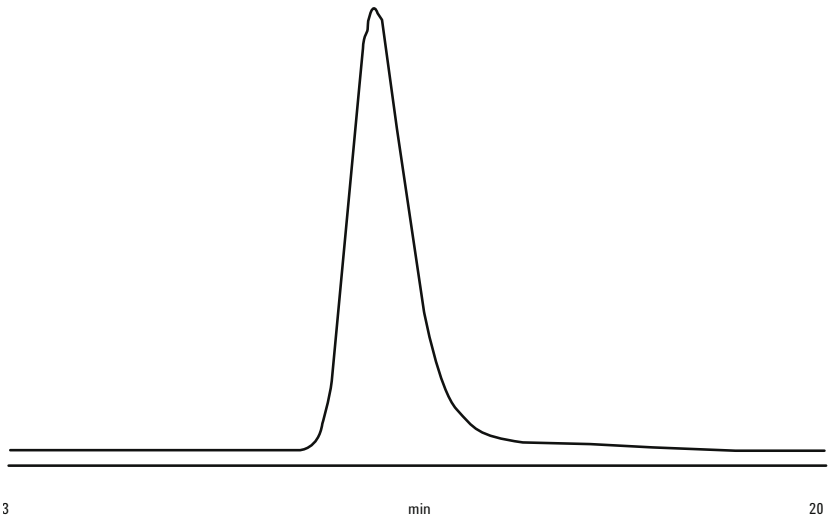


Figure 1. *Typical raw data chromatogram for a poly(lactide-co-glycolide) sample showing a typical gaussian peak shape*

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

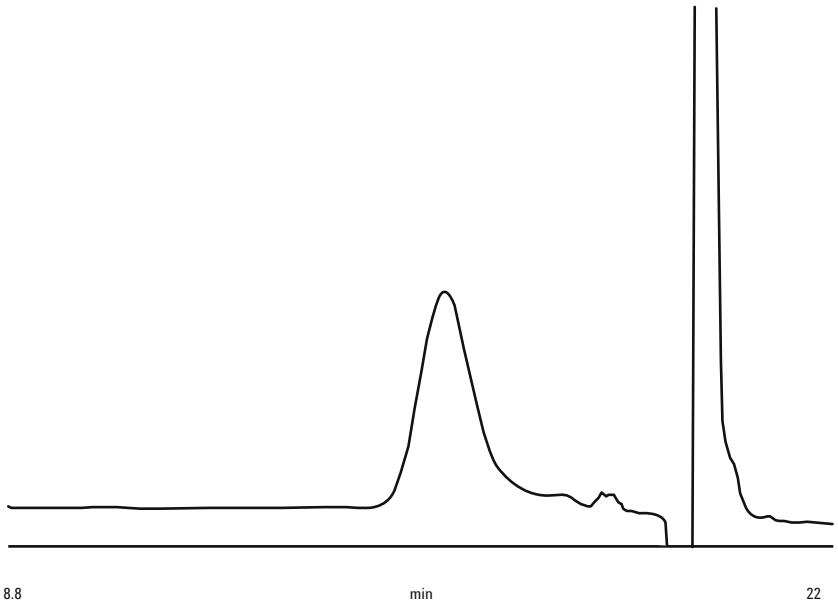


Figure 2. *Typical raw data chromatogram for a sample of polycaprolactam containing low molecular weight components and showing a large system peak*

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

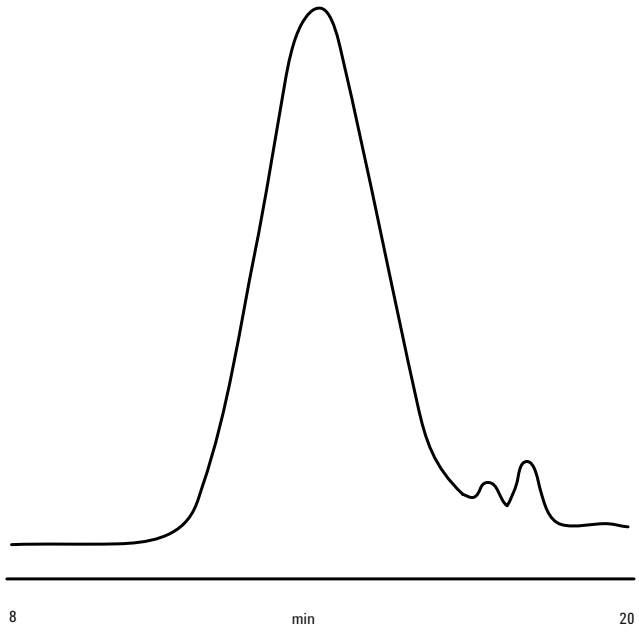


Figure 3. *Raw data chromatogram (sample A) showing the presence of low molecular weight components along with the polymer peak*

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

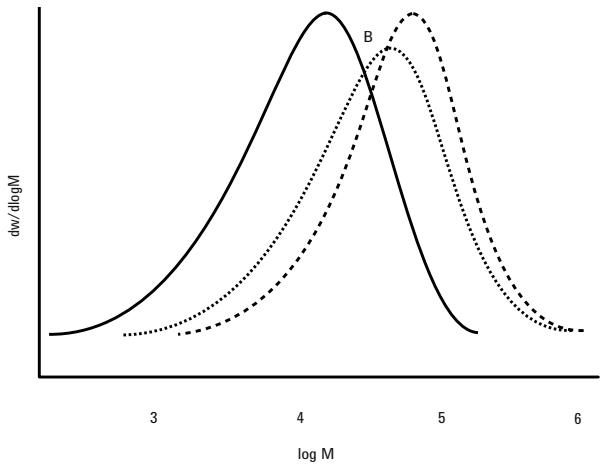


Figure 4. *Example molecular weight distributions of the three polyvinyl alcohols with very different physical properties*

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

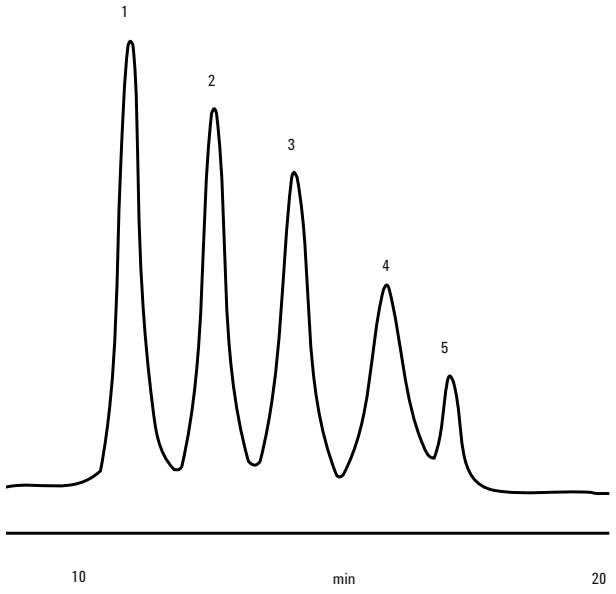


Figure 5. *Resolution achieved in the separation of five PEG samples used for calibration*

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.

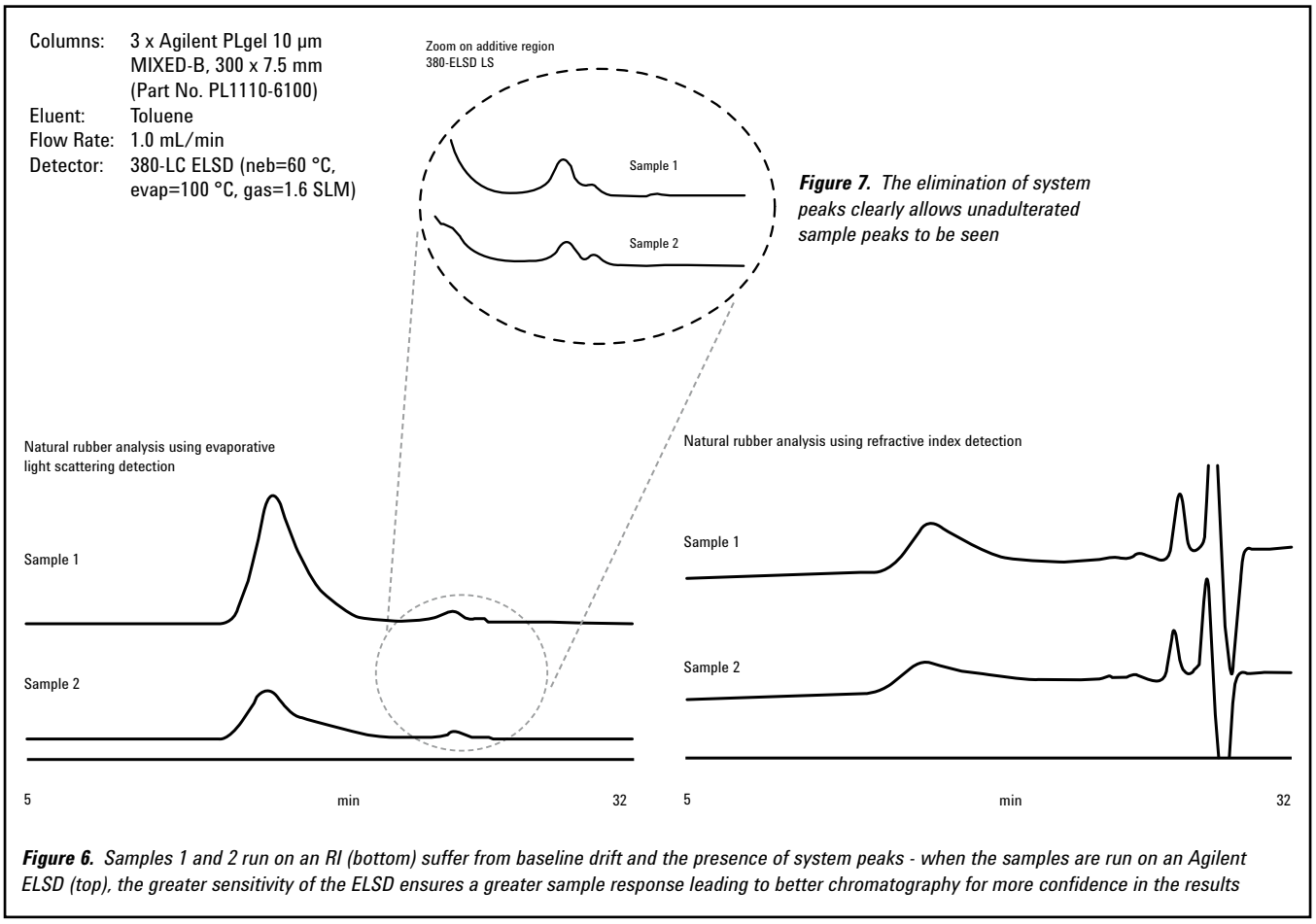


Figure 7. *The elimination of system peaks clearly allows unadulterated sample peaks to be seen*

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

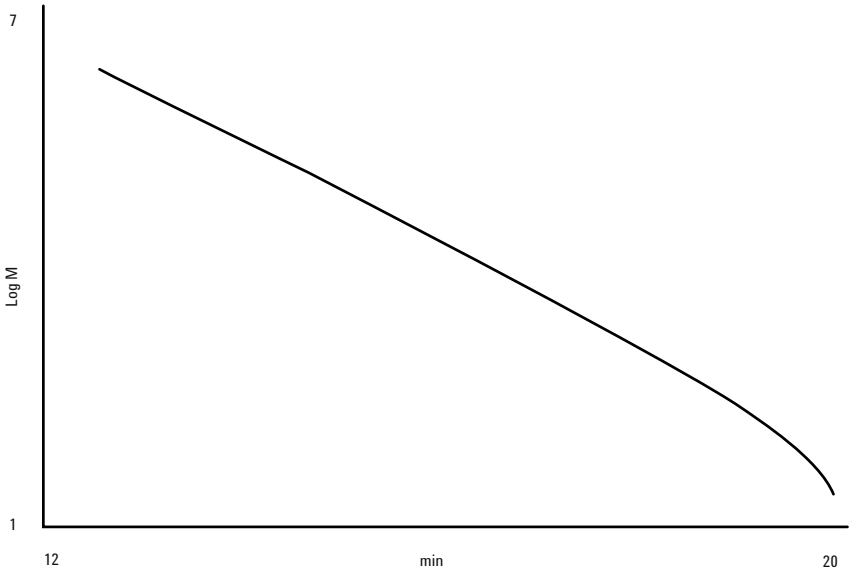


Figure 8. *PL aquagel-OH MIXED-H 8 µm column calibration using PEO EasiVial standards showing the relationship between retention time and the log of molecular weight*

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

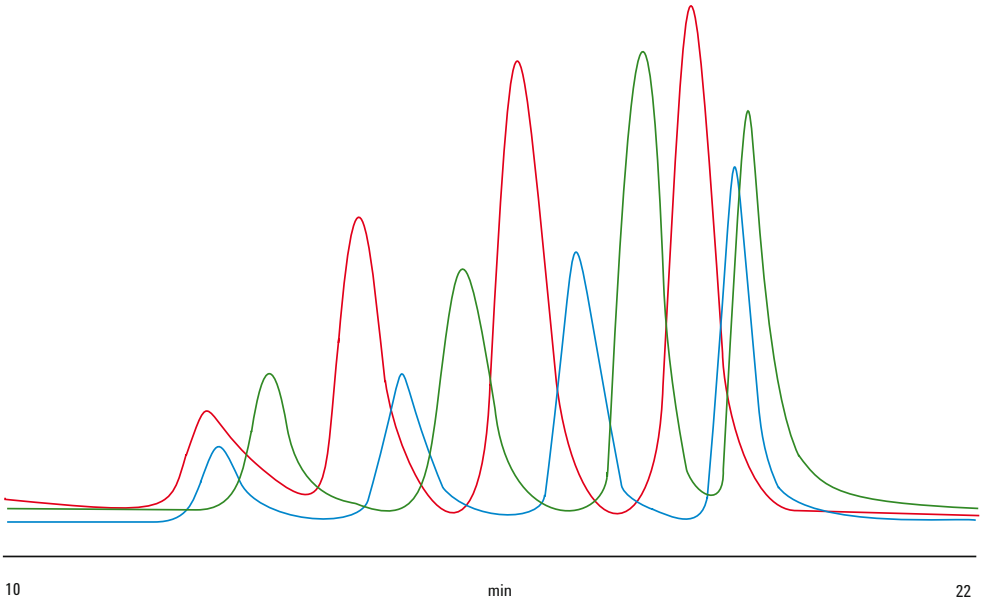


Figure 9. *Example chromatograms of PEO EasiVial standards used to create the calibration*

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

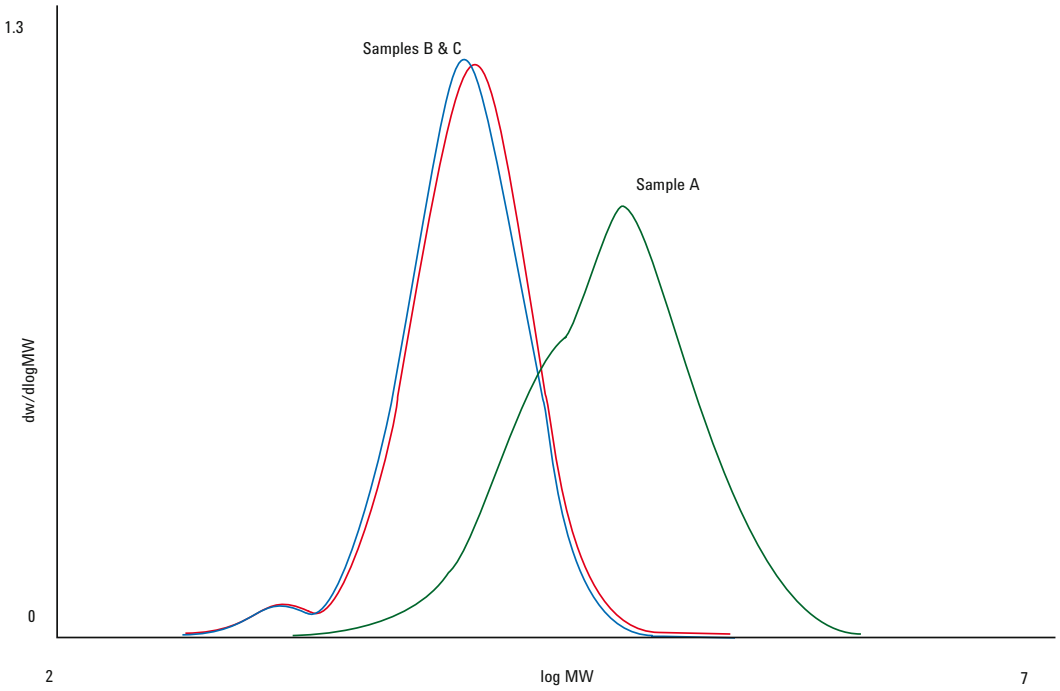


Figure 10. *Molecular weight distribution of three polyacrylic acid samples showing a clear difference between one of the samples (A) and the other two (B and C)*

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

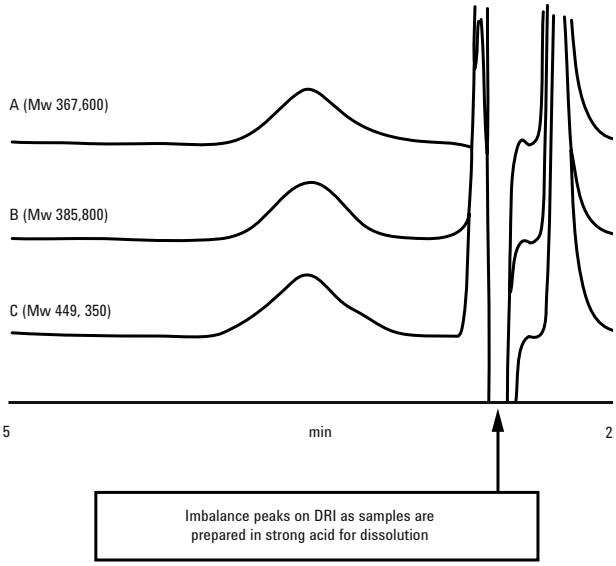


Figure 11. *Raw data chromatograms of three chitosan samples showing typical peak shapes with strong imbalance peaks due to dissolution conditions*

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

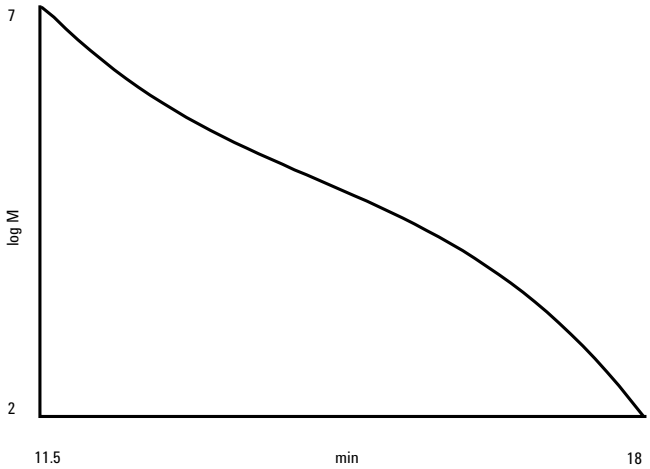


Figure 12. *SEC calibration showing the resolving range of the PL aquagel-OH MIXED-H 8 µm column set*

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

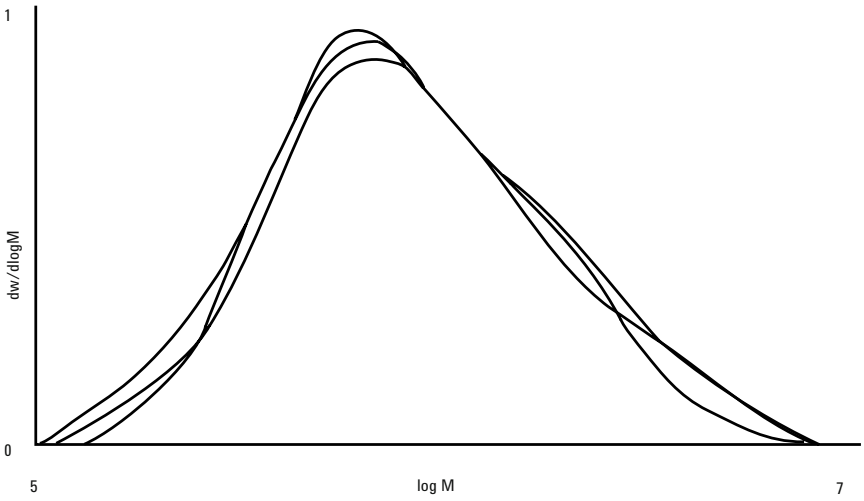


Figure 13. *Example molecular weight distributions of three samples of carboxymethyl cellulose with subtle differences between samples*

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

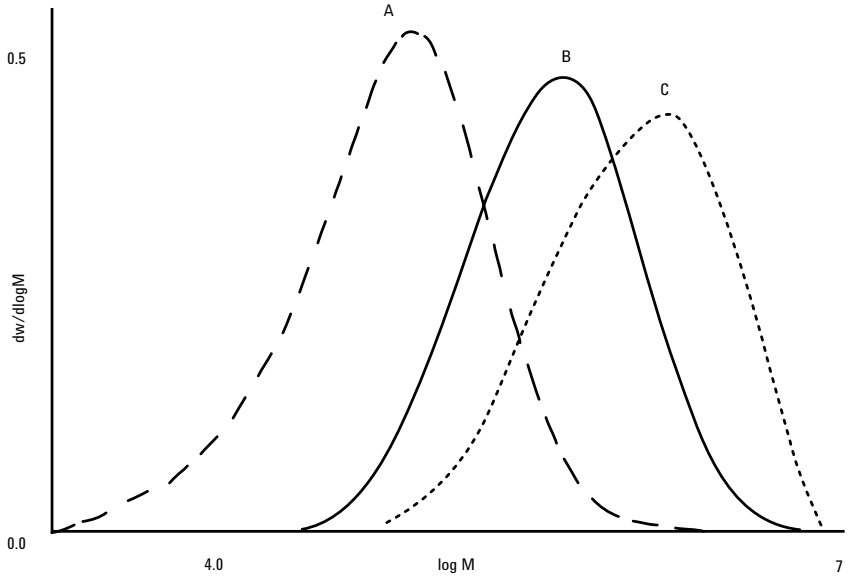


Figure 14. *Example molecular weight distributions of three samples, A, B and C with very different properties*

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

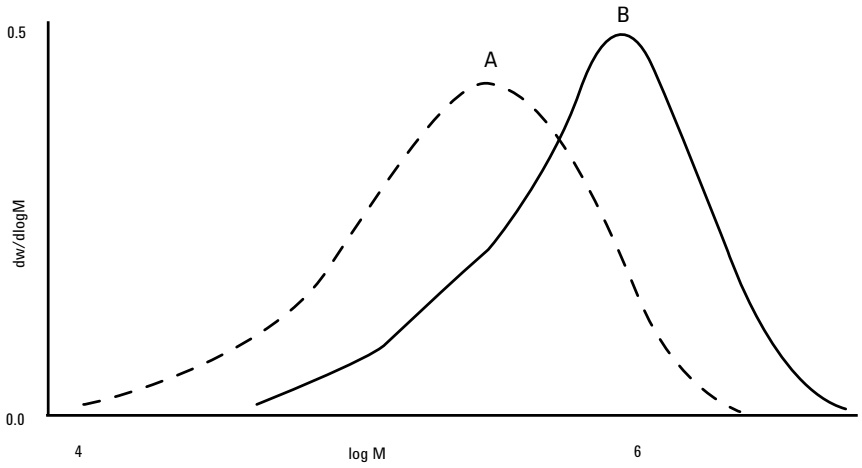


Figure 15. *Example molecular weight distributions for two samples of methyl cellulose which behave very differently*

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)

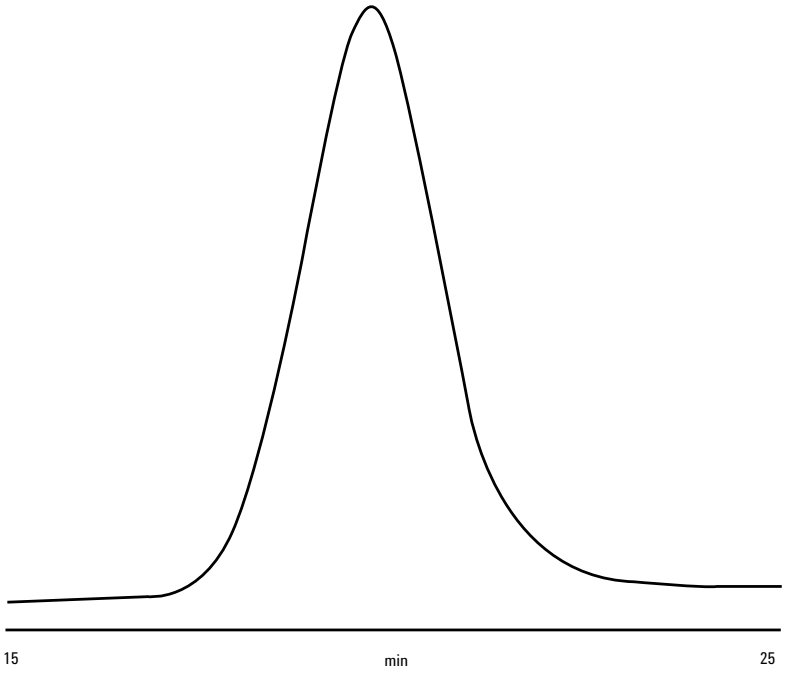


Figure 16. *Example chromatogram for a sample of cellulose acetate analysis showing a typical polymer peak shape*

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

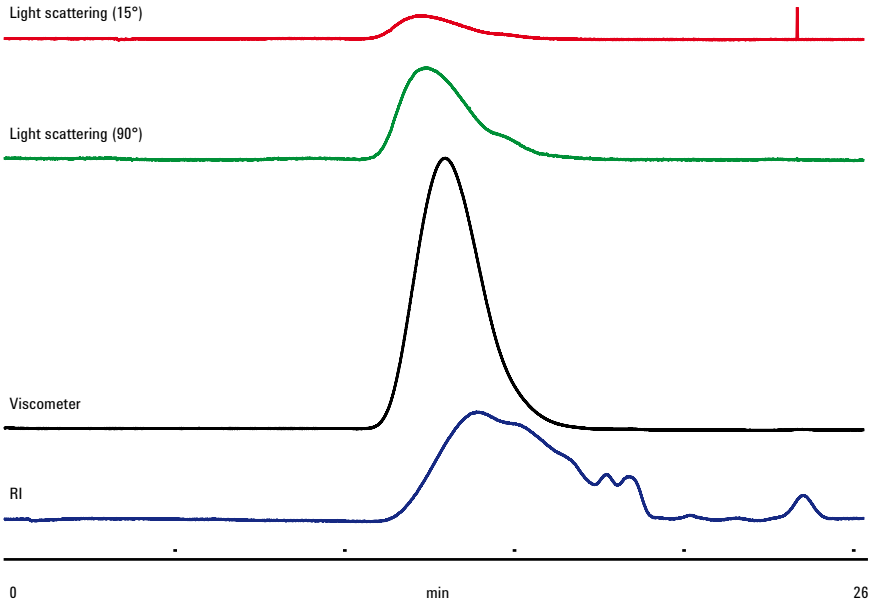
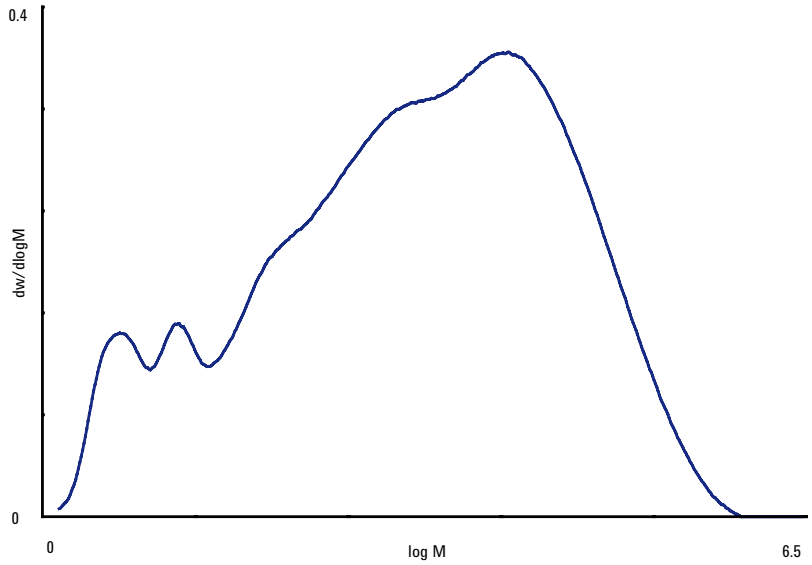


Figure 17. *A typical multi detector overlay of chromatograms for a sample of pectin, showing the different responses of the detector*

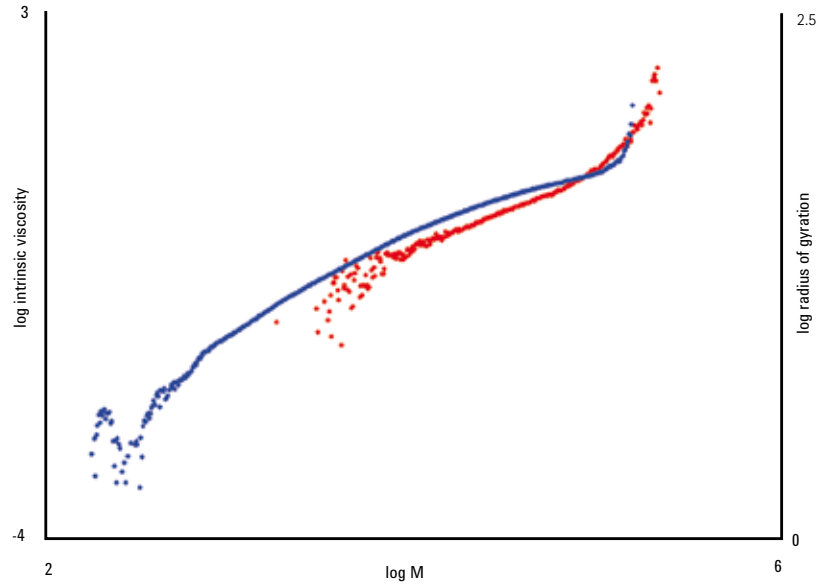
From the viscometry and light scattering data, Mark-Houwink (log intrinsic viscosity versus log M) and conformation (log radius of gyration versus log M) plots were generated for the pectin, shown overlaid in Figure 19.

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*

More Agilent solutions for biodegradable polymers

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Agilent Technologies

SEC Analysis of Pectin

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Pectin is a natural product used extensively as a jellifying, thickening and stabilizing agent in the food industry. It is produced from plant raw materials such as apple, citrus and beet. The extracts are processed to derive pectins with specific properties. Although pectin chemical composition is key to its application, rheological behavior is critical to performance, and determination of the molecular weight distribution can help to predict rheological behavior. SEC and Agilent PL aquagel-OH MIXED-H 8 µm columns are ideal for resolving pectins. With their wide molecular weight resolving range (up to 10 million g/mol relative to PEO/PEG) and high efficiency (>35,000 plates/meter), PL aquagel-OH are the columns of choice for this application.

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Conditions

Pectin samples were prepared at 2 mg/mL, left to fully dissolve overnight and filtered through a 0.45 µm membrane. The column set was calibrated with narrow pullulan standards and, therefore, all molecular weight values quoted are relative to these. The calibration curve is shown is Figure 1.

Samples: Pectin
Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (part number PL1149-6800)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

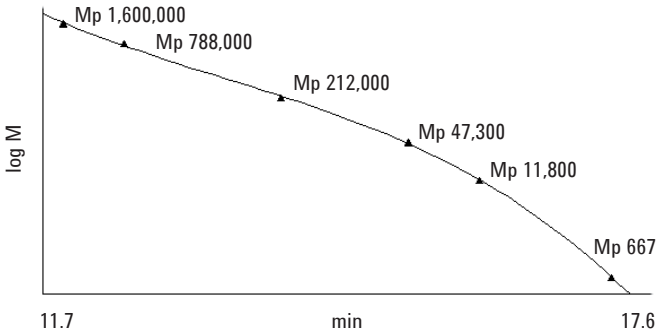

Figure 1. Pullulan standard calibration curve for PL aquagel-OH MIXED-H 8 µm

Results and Discussion

Raw data chromatograms for the pectin samples are illustrated in Figure 2.

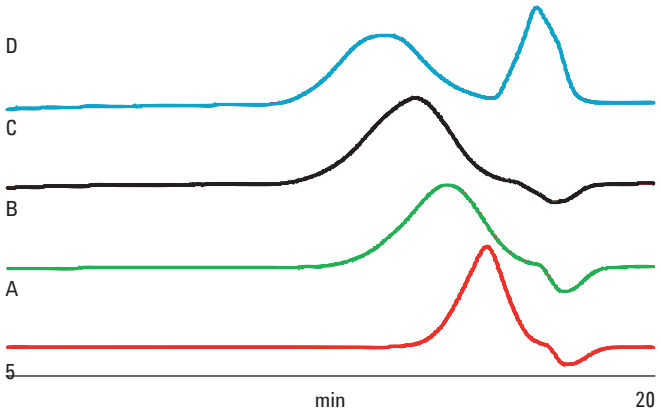

Figure 2. Chromatograms of four pectin samples

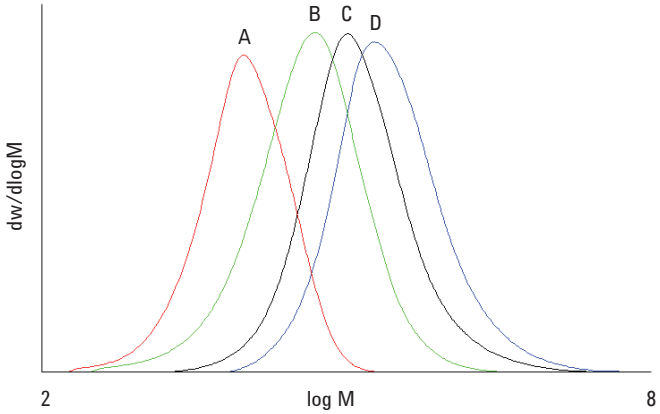

Figure 3. Molecular weight distributions of four pectin samples

Unlike the other samples, sample D exhibits a strong, positive peak around total permeation. This sample is a slow setting grade and contains buffer salts added to modify its properties. Molecular weight averages for the samples are given in the table below.

Table 1. Molecular weight averages for the four pectin samples

Sample	Mn	Mw	Mw/Mn
A	6,520	17,560	2.7
B	21,720	88,480	4.1
C	67,980	243,120	3.6
D	128,360	459,990	3.6

Conclusion

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 pectins reduces interaction between the sample and the columns ensuring that good chromatography is obtained.

www.agilent.com/chem

Published in UK, September 2, 2010
SI-01641

Agilent Technologies

SEC Analysis of Dextrans

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Dextran polysaccharides comprise linked a-D-glucose units with short side chains. These compounds are synthesized in aqueous solutions from fermentation disaccharides produced by lactobacillae such as *Leuconostoc mesenteroides* and *Streptococcus mutans*. In medicine, dextran is used as an antithrombotic to reduce blood viscosity and as an intravenous fluid to solubilize other factors, such as iron, or to replace lost blood in emergencies. In addition, it has a role as a lubricant in some eye drops, and in SEC matrices such as Sephadex. Dextrans are also used as starting or intermediate reagents by food, biotech, photographic and chemical manufacturers.

Size exclusion chomatography will reveal differences in the molecular size profiles of dextrans. Agilent PL aquagel-OH 40 and 60 8 µm columns are ideal for this purpose because they combine low exclusion limit, high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. In this case, two different PL aquagel-OH columns were connected in series to cover a molecular weight range from 10^4 to 10^7.

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Conditions

Samples: Four dextrans
Columns: 2 x PL aquagel-OH 60 8 µm, 300 x 7.5 mm (p/n PL1149-6860 + 1 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.2 M NaH_2PO_3 + 0.2 M NaCL at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Figure 1 shows the differences in molecular weights of four commercial dextrans.

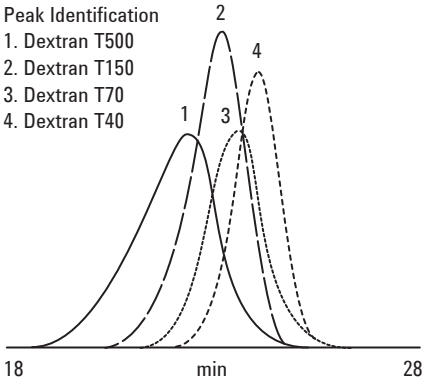

Figure 1. Overlay chromatograms showing the differences in molecular weights of four commercial dextrans

Conclusion

SEC and PL aquagel-OH columns successfully resolved four samples of dextran. The 'neutral' surface and ability to operate across a wide range of eluent conditions equip PL aquagel-OH for the high performance analysis of analytes with neutral, ionic and hydrophobic moieties, singly or combined.

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SI-01630

Agilent Technologies

Identification of Unknown Reaction By-Products and Contaminants in Epoxyphenolic-Based Food Can Coatings by LC/TOF-MS

Application

Food Safety

Authors

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Abstract

This application illustrates how time-of-flight mass spectrometry can be used in the safety evaluation of new and existing can coatings used in the food industry. The accurate mass provides information for the parent compound and fragment ions greatly increase the confidence in the identification process.

Introduction

The internal surface of metal cans used to pack foodstuffs is often coated to form a barrier between the food and the metal of the can. The coating formulation may contain various components such as resins, crosslinking agents, catalysts, lubricants, wetting agents, and solvents. The potential exists for these ingredients, or by-products of reactions between them, to migrate from the can coating into foods. Thus existing and especially new coatings need to be evaluated for their safety for contact with food and beverages.

We will illustrate this evaluation using the example of epoxyphenolic can coatings based on bisphenol A epoxy resins. These are cured by stoving with phenolic resins to produce a three-dimensional crosslinked network to provide the chemical and pack resistance required for food and beverage cans. The epoxy monomer bisphenol A diglycidyl ether (BADGE, see Figure 1) participates in these polymerization reactions via its reactive epoxide groups. However, it can also undergo addition from attacking nucleophiles such as water or solvents to give lower molecular weight products that might migrate into the packed food [1–3]. These potential migrants need to be identified.

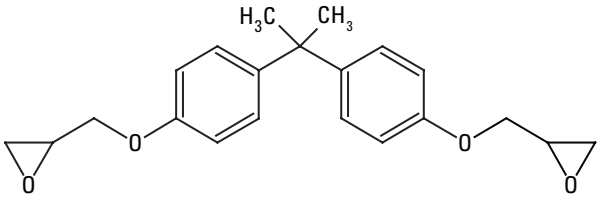

Figure 1. Chemical structure of BADGE, $C_{21}H_{24}O_4$.

The accurate mass measurements provided by time-of-flight (TOF) mass spectrometry (MS) for unknown compounds makes this identification process possible without the need for authentic standards of every possible minor impurity and reaction by-product.

Experimental

Sample Extraction

A metal panel (250 cm^2) coated with an epoxyphenolic lacquer and stoved under industrial conditions was cut into pieces (approximately 1 cm^2) and extracted by immersion in acetonitrile (100 mL). After 18 hours the extract was evaporated to a small volume (1 mL).

LC Conditions

Instrument:	Agilent LC 1200 SL
Mobile phases:	A: water B: acetonitrile
Gradient:	20% B to 50% B over 25 min, hold 20 min, 100% B at 60 min, hold 10 min, return to 20% B over 10 min
Flow rate:	0.2 mL/min
Column:	Agilent ZORBAX Eclipse XDB, 100 mm × 2.1 mm, 3.5-µm particles Part number 961753.902
Injection:	5 µL

MS Conditions

Instrument:	Agilent 6210 LC/MS TOF in positive ion ESI mode
Nebulizer press.:	30 psi
Capillary:	4000 V
Gas temp.:	300 °C
Drying gas:	7 L/min

Results and Discussion

TOF-MS parameters were optimized using solvent standards of BADGE, as mainly BADGE derivatives were expected to be extracted from the coating [1]. A fragmentor value of 150 V was used first, to cause no fragmentation, and so molecular ion adducts were seen. Figure 2 shows the TIC for the acetonitrile extract of the epoxyphenolic coating. There are many unknown peaks, and the one at 27.2 min was chosen for this example.

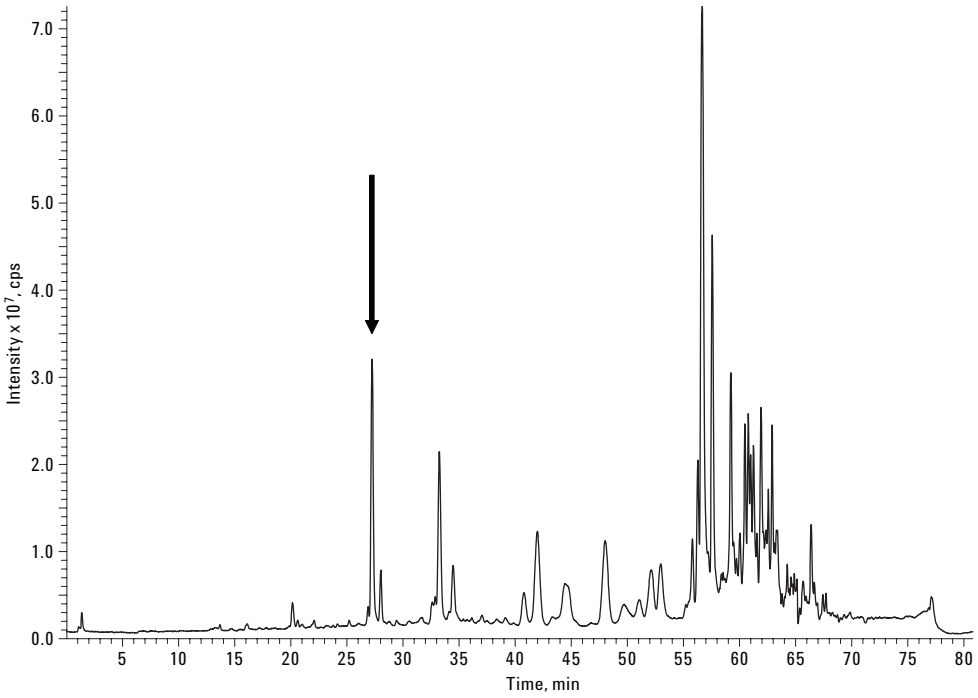

Figure 2. Total ion chromatogram of the acetonitrile extract of the epoxyphenolic coating.

Figure 3 shows the mass spectrum of the peak at 27.2 min. The differences in masses between the ions suggest that these are due to the protonated, ammoniated, sodiated, and potassiated molecule. No ammonia, sodium, or potassium was added to the mobile phase, and it is likely that these adducts arose due to contamination from other work carried out on the instrument, or were present in the solvents used in the mobile phase.

The formula calculator was used to propose identities for the peak, using the accurate mass determined for [M+NH4]+, as it was the most intense. Only one possible empirical formula was provided limiting the elements to C, H, O, and only one N within the 5 ppm mass error limit used.

For the experimentally derived mass 494.3118, the formula $C_{27}H_{44}O_7N$ was proposed (theoretical mass 494.3112, 1.15 ppm error). As it is proposed that this is the ammoniated adduct (subtract NH_4), this gives a formula of $C_{27}H_{40}O_7$ for the unknown peak. Furthermore, it is suspected that this peak is a BADGE derivative (subtract $C_{21}H_{24}O_4$ from the formula) and this suggests that the unknown peak is BADGE + $C_6H_{16}O_3$.

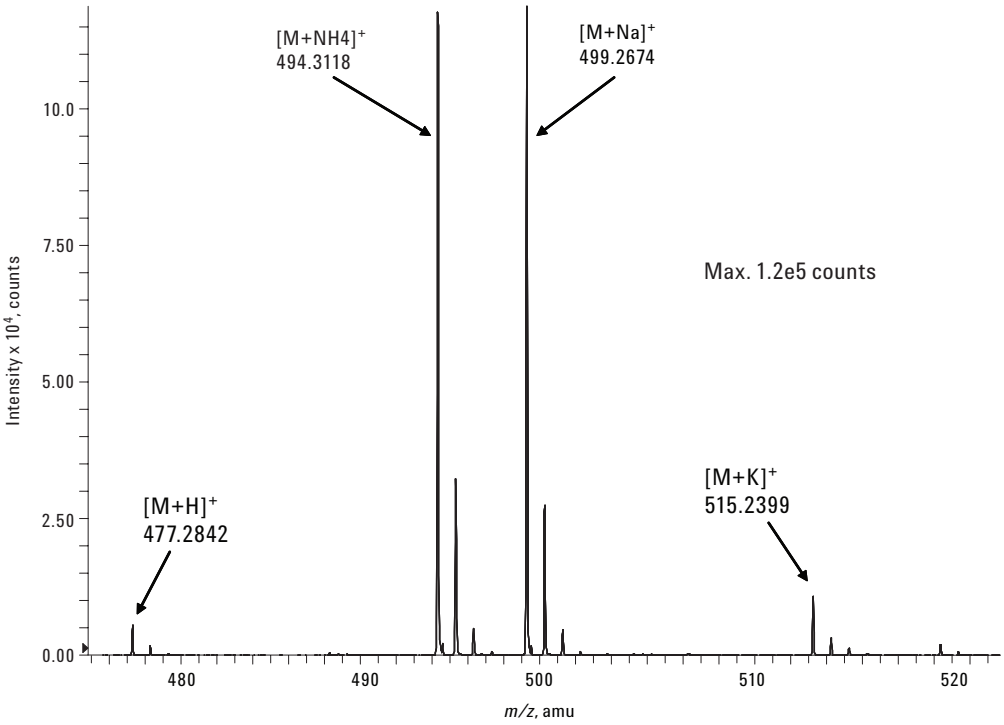

Figure 3. Mass spectrum of the unknown peak at 27.2 min (fragmentor = 150 V).

Fragmentation experiments were carried out to aid the identification process. A fragmentor value of 275 V dissociated the ammoniated molecular adduct into fragment ions, see Figure 4.

The accurate masses of the fragment ions were put into the formula calculator and the structures of the ions were theorized from the proposed empirical formulae. The fragment ions confirmed the presence of the BADGE unit (*m/z* 341.1727), that one of the epoxide rings had reacted with water (fragment ion at *m/z* 209.1149), and the other had reacted with butoxyethanol (BuOEtOH, $C_6H_{16}O_3$) (fragment ion at *m/z* 309.2036), a solvent used in the manufacturing process of the coating formulation. Figure 5 shows the structure of BADGE.H_2O.BuOEtOH.

Using the same approach, the identity of virtually all of the peaks in Figure 2 was established and different can coating chemistries have been studied.

Conclusions

Solvent extracts of epoxyphenolic can coatings have been analyzed by LC/TOF-MS to identify potential migrants into food and beverages. Accurate mass data of the parent compound and the fragment ions allows confident assignment of previously unknown peaks. Using the LC/TOF-MS has helped the testing of existing can coatings and guided the development of new coating chemistries.

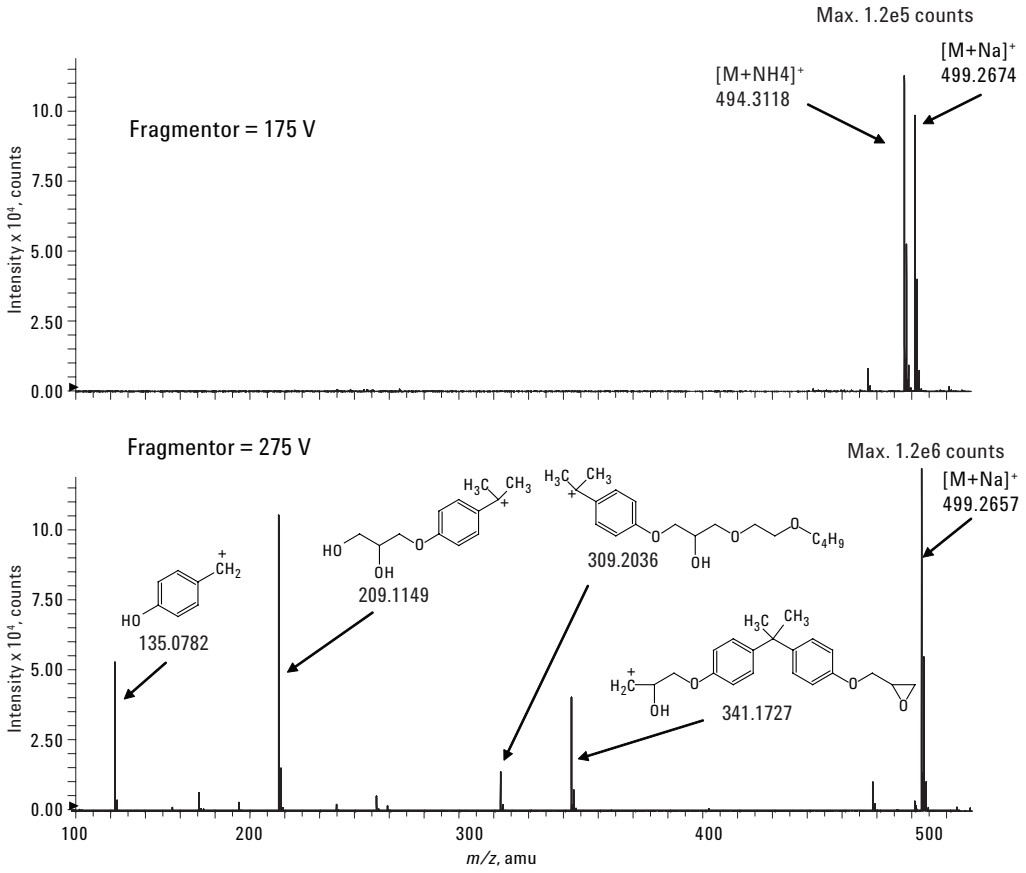

Figure 4. TOF-MS of the unknown peak at 27.2 min.

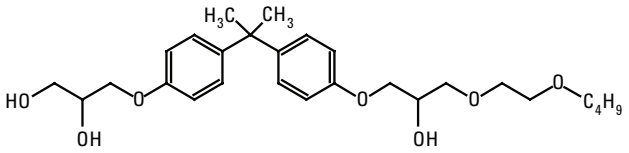

Figure 5. Structure of the identified compound: BADGE.H_2O.BuOEtOH.

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2. N. Leepipatpiboon, O. Sae-Khow, and S. Jayanta (2005) *Journal of Chromatography A*, 1073, 1–2, 331-339.
3. O. Pardo, V. Yusa, N. Leon, and A. Pastor (2006) *Journal of Chromatography A*, 1107, 1–2, 70–78.

Acknowledgements

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Agilent Technologies

Identification of Unknown Polyester Oligomers in New Polyester Food Can Coatings by LC/TOF-MS Using Molecular Feature Extraction and Database Searching

Application

Food Safety

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Abstract

This application illustrates how time-of-flight mass spectrometry (TOF-MS) can help in the safety evaluation of new and existing polyester-based coatings used on the inside of food cans. A database of over 1,000 possible migrants based on small polyester oligomers that may not be incorporated into the polymer network of the coating was prepared from likely starting materials and the exact mass of each one was calculated. Automated database searching using the Agilent Data Analysis software and molecular feature extraction (MFE) compared the accurate mass information provided by the TOF-MS analysis to the database to identify previously unknown chromatographic peaks.

Introduction

The development of new and improved internal coatings for food and beverage cans must be cognizant of the legislative requirements on food contact materials [1]. If not, otherwise promising developments with good technical performance could come to a wasteful dead end. Coating formulations usually contain various components such as resins, cross-linking agents, catalysts, lubricants, wetting agents, and solvents. The potential exists for these ingredients, or by-products of reactions between them, to migrate from the can coating into foods. Thus, existing and especially new coatings must be evaluated for their safety for contact with food and beverages.

An earlier application described the analysis of a can coating based on epoxy resins [2]. Polyester-based coatings provide an alternative to epoxy resins and in these systems the three-dimensional polymer network is built up from a number of possible poly-functional alcohol and carboxylic acid monomers [3]. Oligomers are by-products of the polymerization process and can migrate from the coating into the food [4]. These oligomers can be formed from all possible combinations of the monomers used to construct the polymer. Some of the most common monomers used to prepare polyester resins are given in Table 1.

Combination of the different monomers provides a large number of possible polyester oligomers with the potential to migrate from the can coating into food. The accurate mass measurements provided by TOF-MS makes the identification of potential migrants possible without the need for authentic standards of every possible polyester oligomer. The Agilent MFE data analysis tool allows very fast searching of the unknown chromatographic peaks against a large user-prepared database of possible polyester oligomers.

Experimental

Sample Extraction

A metal panel (250 cm^2) coated with a polyester lacquer and stoved under industrial conditions was cut into pieces (approximately 1 cm^2) and extracted by immersion in acetonitrile (100 mL). After 18 h the extract was concentrated to a small volume (1 mL) for LC/TOF-MS analysis.

LC Conditions

Instrument	Agilent 1200 SL
Column	Agilent ZORBAX XDB-C18 100 mm × 2.1 mm, 3.5 µm Agilent p/n: 961753-902
Mobile phases	A: Water B: Acetonitrile
Gradient	20% B to 50% B over 25 min, hold 20 min, 100% B at 60 min, hold 10 min, return to 20% B over 10 min
Flow rate	0.2 mL/min
Injection volume	5 µL

MS Conditions

Instrument	Agilent 6210 TOF-MS in positive ion ESI mode
Nebulizer pressure	30 psi
Capillary:	4000 V
Gas temperature	325 °C
Drying gas	10 L/min

Data Analysis (DA) Parameters

DA operation	Molecular Feature Extraction	
Report type	Include confirmation screening by database search	
Processing option/peak detection		
	S/N threshold	50
	Minimum relative volume	2.5%
Adduct	H, NH_4, Na, K	
Confirmation screen		
	Mass tolerance	5 ppm

Results and Discussion

Figure 1 shows the total ion chromatogram (TIC) of the polyester coating extract. Although the amounts detected are low, these peaks need to be identified for a complete safety evaluation of the coating intended for food cans.

Because an unknown number of different polyols and polyacids can be used to make polyester resins (see Table 1 for some common examples), many polyester oligomers are theoretically possible. An Excel spreadsheet was prepared to calculate the exact mass of all possible oligomers and this contained over 1,000 possibilities [2,3]. An excerpt is given in Table 2. Although this is seemingly a very cumbersome and time-consuming process, once the database is constructed it allows very rapid and efficient data analysis and identification of unknown peaks in all further polyester-based coatings analyzed. The Excel spreadsheet was converted to .CSV format and used with the Agilent Data Analysis software to automatically search the chromatogram of unknown peaks (Figure 1) and compare it to the oligomer masses. Use of MFE, even with over 1,000 entries, makes this process very fast.

Table 3 gives the identities of the 13 polyester oligomers detected and identified using the MFE software. The confidence in the identification is good as all mass error values (difference between the measured and theoretical masses) are less than 5 ppm. Analysis revealed the polyester was based on phthalic acids esterified with five of the polyols listed in Table 1. Twelve of the 13 oligomers are cyclic and only one is linear. This is reasonable because cyclic oligomers cannot be incorporated into the polymeric network of the coating, making them more readily available to migrate.

The TIC (Figure 1) shows more peaks than the 13 polyester oligomers identified in Table 2. This is due to isomeric forms of the oligomers that chromatograph differently. These can arise from (a) different isomers of the starting substances (for example, ortho-, meta-, or para-phthalic acid, or isomeric polyols); (b) oligomers having the same composition but different structures (for example, linear PA+EG+PA+EG+PA+NPG versus linear PA+EG+PA+NPG+PA+EG); (c) two or more oligomers having the same empirical formula but a different identity (for example, 3PA+2EG+NPG and 3PA+3PG both have the formula $C_{33}H_{30}O_{12}$); (d) diastereoisomers formed when incorporating the chiral 1,3-propylene glycol monomer.

The identity of those peaks not assigned as polyester oligomers were proposed based on chemical knowledge and analysis of the starting materials for the lacquer. One of these was proposed to be a plasticizer found in a starting material, three were from lubricants (two found in starting materials) and two from surfactant-type molecules (not found in starting materials).

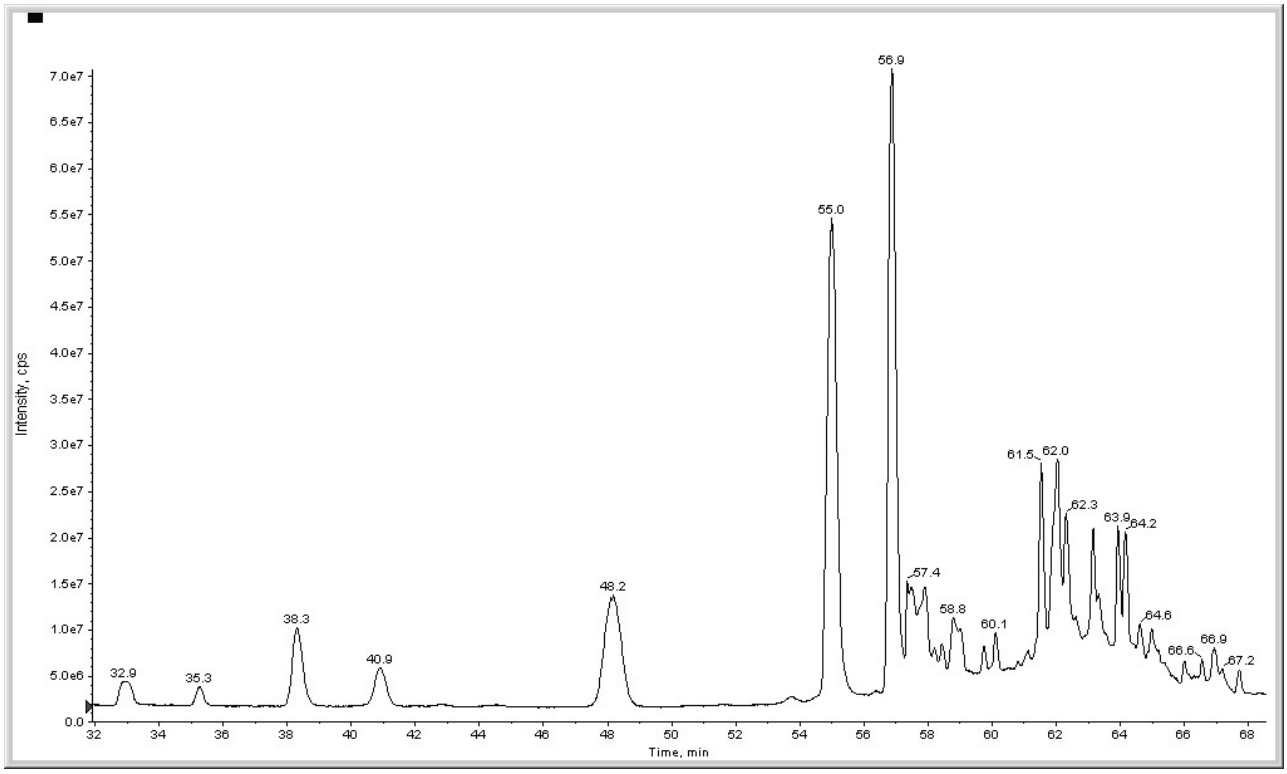

Figure 1. Total ion chromatogram (TIC) of the polyester coating extract.

Table 1. Commonly Used Monomers for Polyester Resins [2,3]

Common name	Abbreviation	Formula
Polyols		
Ethylene glycol	EG	$C_2H_6O_2$
Propylene glycol (1,2- and 1,3-)	PG	$C_3H_8O_2$
Butanediol (1,3- + isomers)	BD	$C_4H_{10}O_2$
Diethylene glycol	DEG	$C_4H_{10}O_4$
Neopentyl glycol	NPG	$C_5H_{12}O_2$
1,6-Hexanediol	HD	$C_6H_{14}O_2$
Tris(hydroxymethyl)propane	HMP	$C_6H_{14}O_3$
Cyclohexyldimethanol	CHDM	$C_8H_{16}O_2$
2,2,4-Trimethylpentane-1,3-diol	TMP	$C_8H_{18}O_2$
Polyacids		
Adipic acid	AA	$C_6H_{10}O_4$
Phthalic acid (o, m, and p-isomers)	PA	$C_8H_6O_4$
Trimellitic acid	TMA	$C_9H_6O_6$

Table 2. Excerpt from the Polyester User-Prepared Database (See Table 1 for Abbreviations)

		AA	TMA	PA	CHDM	BD	EG	DEG	PG	HD	HMP	TMP	NPG	H_2O	MW
PA+EG	Linear			1			1							1	210.0528
EG+PA+EG	Linear			1			2							2	254.0790
PA+EG+PA+EG	Linear			2			2							3	402.0951
PA+EG+PA+EG	Cyclic			2			2							4	384.0845
PA+EG+PA+EG+PA	Linear			3			2							4	550.1111
PA+EG+PA+EG+PA+NPG	Linear			3			2						1	5	636.1843
PA+EG+PA+NPG+PA+EG	Linear			3			2						1	5	636.1843
PA+PG+PA+PG+PA+PG	Linear			3					3					5	636.1843
PA+PG+PA+PG+PA+PG	Cyclic			3					3					6	618.1737

Table 3. Polyester Oligomers Identified Using Molecular Feature Extraction and Database Searching

Mass of compound	Formula predicted	Mass error (ppm)	Proposed identity	Notes
384.0845	$C_{20}H_{16}O_8$	1.4	2PA+2EG	Cyclic
426.1315	$C_{23}H_{22}O_8$	1.4	2PA+EG+NPG	Cyclic
428.1107	$C_{22}H_{20}O_9$	1.1	2PA+EG+DEG	Cyclic
466.1630	$C_{26}H_{26}O_8$	0.36	2PA+CHDM+EG	Cyclic
468.1784	$C_{26}H_{28}O_8$	0.50	2PA+2NPG	Cyclic
508.2114	$C_{29}H_{32}O_8$	3.3	2PA+CHDM+NPG	Cyclic
618.1737	$C_{33}H_{30}O_{12}$	0.84	3PA+2EG+NPG or 3PA+3PG	Cyclic
660.2238	$C_{36}H_{36}O_{12}$	4.6	3PA+EG+2NPG	Cyclic
700.2520	$C_{39}H_{40}O_{12}$	0.82	3PA+CHDM+EG+NPG	Cyclic
702.2703	$C_{39}H_{42}O_{12}$	4.8	3PA+3NPG	Cyclic
704.2469	$C_{38}H_{40}O_{13}$	0.79	3PA+CHDM+2PG	Linear
742.3003	$C_{35}H_{50}O_{17}$	0.74	3PA+CHDM+2NPG	Cyclic
782.3330	$C_{45}H_{50}O_{12}$	4.2	3PA+2CHDM+NPG	Cyclic

Conclusions

Solvent extracts of polyester can coatings have been analyzed by LC/TOF-MS to identify potential migrants into food and beverages. Accurate mass data of the parent compounds and automated data analysis software with MFE allowed confident assignment of previously unknown peaks by searching a user-prepared database of possible polyester oligomers. The database allows rapid identification of these oligomers in many complex samples. In this work LC/TOF-MS has helped to ensure the safety of food can coatings and has guided the development of new coating chemistries.

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LC-TOF-MS As a Tool to Support Can Coating/Food Interaction Studies

Application

Food Safety

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Abstract

This application illustrates how time-of-flight mass spectrometry has been used in the studies of interactions at the can coating/food interface of internally coated metal cans intended for use in the food industry. Previously unconfirmed migrants were confidently identified using accurate mass information provided.

Introduction

The internal surface of metal cans used to pack foodstuffs is often coated to form a barrier between the food and the metal of the can. The coating formulation may contain various components, such as resins, cross-linking agents, catalysts, lubricants, wetting agents, and solvents. The potential exists for these ingredients, or by-products of reactions between them, to migrate from the can coating into foods.

Food ingredients such as fat or water can cause some coatings to swell, which may enhance any migration, particularly if the food is heat processed in its packaging. Migration can also depend on other factors: contact time and temperature, the type and thickness of the coating, and the molecular mass and size of the migrating species. Studies, in particular migration modeling, of interactions between the can coating and the foodstuff are important in understanding, and eventually reducing, migration of compounds from the can coating into the foodstuffs.

In previous applications we have described the analysis of can coatings based on epoxy resins [1] and polyester resins [2] and how the accurate mass information for the parent compounds and fragment ions greatly increased the confidence in the identification of unknown compounds. In this application we describe how liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) was used as a tool to support studies on can coating/food interactions

Experimental

Coated Panels

The epoxyphenolic (EPH) lacquer was applied to metal panels and cured in an oven at 200 °C for 10 min. Small test specimens with an area of 9 cm^2 were cut from the panel and folded to a concertina shape ready for migration studies.

Exposure to Sunflower Oil

The folded test specimens of coated panel were submerged in sunflower oil in a pressurised vial at 121 °C in a silicon oil bath for 10, 20, 30, 40, 50, 60, 70, and 120 min. After exposure, the coated

metal test specimens were wiped to remove the oil and submerged in acetonitrile (25 mL) overnight. A portion of the acetonitrile (1 mL) was passed through a 0.2-mm PTFE filter.

Hydrochlorination

Concentrated hydrochloric acid (100 µL) was added to a portion (500 µL) of the concentrated (10 times) EPH acetonitrile extract. This was allowed to react at 60 °C for 18 h in a sealed vial and made up to 1 mL with acetonitrile.

Liquid Chromatography-Fluorescence Detection (LC-FLD)

Instrument:	Agilent 1200 Series LC and G1321A FLD
Mobile phases:	A = 0.1% acetic acid in water B = acetonitrile
Gradient:	At t = 0, B = 35%; t = 5 min, B = 50%; t = 10 min, B = 50%; t = 20 min, B = 100%; t = 25 min, B = 100%
Flow rate:	1.0 mL/min
Column:	Agilent ZORBAX Eclipse XDB, 100 mm × 2.1 mm, 3.5-µm particle size (part number 961753.902)
Injection:	20 µL
Excitation wavelength:	275 nm
Emission wavelength:	305 nm
Gain:	2^10

LC-FLD-TOF-MS

Instrument:	Agilent 1200 Series LC, G1321A FLD and TOF positive electrospray
Mobile phases:	A = 0.1% acetic acid in water B = acetonitrile
Gradient:	At t = 0 min, B = 35%; t = 5 min, B = 50%; t = 20 min, B = 50%, t = 30 min, B = 100%; t = 40 min, B = 100%
Flow rate:	0.3 mL/min
Column:	Agilent ZORBAX Eclipse XDB, 100 mm × 2.1 mm, 3.5-µm particle size (part number 961753.902)
Injection:	5 µL
Excitation wavelength:	275 nm
Emission wavelength:	305 nm
Gain:	2^10
Nebulizer pressure:	30 psi
Capillary:	4000 V
Gas temperature:	325 °C
Drying gas:	10 L/min
Fragmentor:	150 V

Acylation

A method reported by Biedermann and Grob was adapted for use [3]. A portion (5 mL) of the EPH acetonitrile extract was evaporated to dryness under nitrogen. Acetic anhydride (25 µL) and pyridine (25 µL) were added and allowed to react for 15 min. The excess reagents were removed by evaporation under nitrogen and the residue was redissolved in acetonitrile (500 µL).

Another portion (5 mL) of EPH acetonitrile extract was evaporated under nitrogen and treated with acetic anhydride as described above. After removal of the excess reagents by evaporation, trifluoroacetic acid (TFAA, 100 µL) was added. This was allowed to react for 15 min and the excess reagent removed by evaporation under nitrogen. The dry residue was redissolved in acetonitrile (500 µL).

Results and Discussion

During migration tests using panels coated in a generic EPH coating, two co-eluting peaks were studied by LC-FLD. The identity of a pair of peaks at a similar retention time has been previously reported in the literature as two isomers of cyclo-di-BADGE [4]. The structure of cyclo-di-BADGE is shown in Figure 1.

The EPH coated panels were exposed to sunflower oil at 121 °C for increasing periods of time and the co-eluting peaks were seen to behave differently, as shown in Figure 2. The profile of the two peaks, 2-1 and 2-2, was seen to change: as the length of exposure increased, the relative peak height of peak 2-1 decreased compared to that of peak 2-2, suggesting that this compound was migrating at a greater

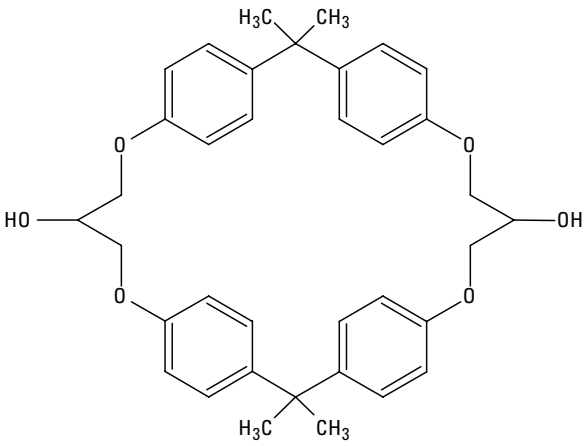

Figure 1. Structure of cyclo-di-BADGE, $C_{36}H_{40}O_6$.

rate. After 120 min, the two peaks had approximately equal peak heights.

The identification power of TOF-MS shown in previous applications [1-2] was used to investigate the EPH extracts with the aim of confidently identifying the co-eluting peaks and explaining the migration behavior seen. The flow rate of 1.0 mL/min used in the LC-FLD was deemed too high to be used on the LC-TOF-MS (although this ESI source can accept a flow rate of 1.0 mL/min), so the LC conditions were adapted. The LC-TOF-MS apparatus had a FLD in series. Figure 3 shows the FLD chromatogram of a concentrated acetonitrile EPH extract. The slower gradient means that the peaks are now eluting later (22.4 min and 23.15 min) and there are now three peaks, because the extra time on the column has allowed greater interactions with the stationary phase, which in turn has allowed greater resolution between the peaks.

This becomes clearer when looking at the TOF-MS data for this chromatogram (Figure 4). There are three peaks: 4-1 at 22.13 min, 4-2 at 22.61 min, and 4-3 at 23.45 min. Peaks 4-2 and 4-3 have the same mass spectra and molecular formula ($C_{36}H_{40}O_5$), as shown in Figure 5 and Table 1. Peak 4-1 has a different molecular formula ($C_{25}H_{34}O_5$).

From this data the molecular formula of peak 4-1 was proposed as $C_{25}H_{34}O_5$ with an identity of BADGE.BuOH. This identity was deduced based on methods reported in an earlier application [1]. The identity was confirmed by the addition of HCl to the extract. As expected, the BADGE.BuOH peak disappeared and was replaced by a peak with a

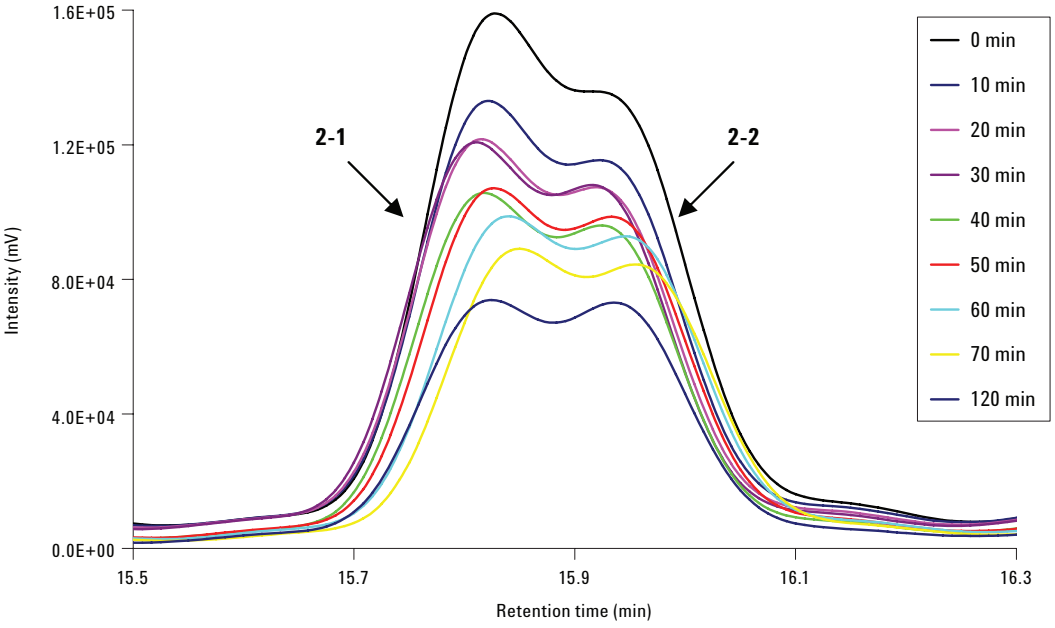

Figure 2. LC-FLD chromatogram obtained after exposing the EPH can coating to sunflower oil for different lengths of time.

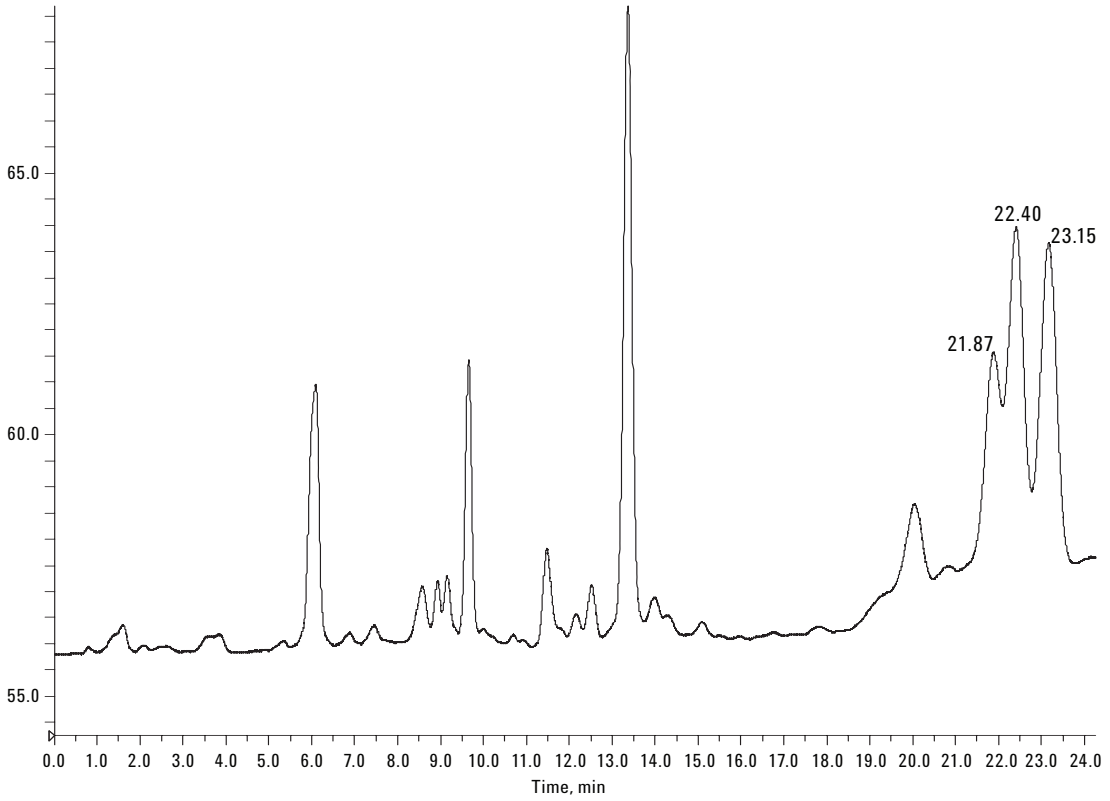

Figure 3. LC-FLD chromatogram of the concentrated EPH acetonitrile extract.

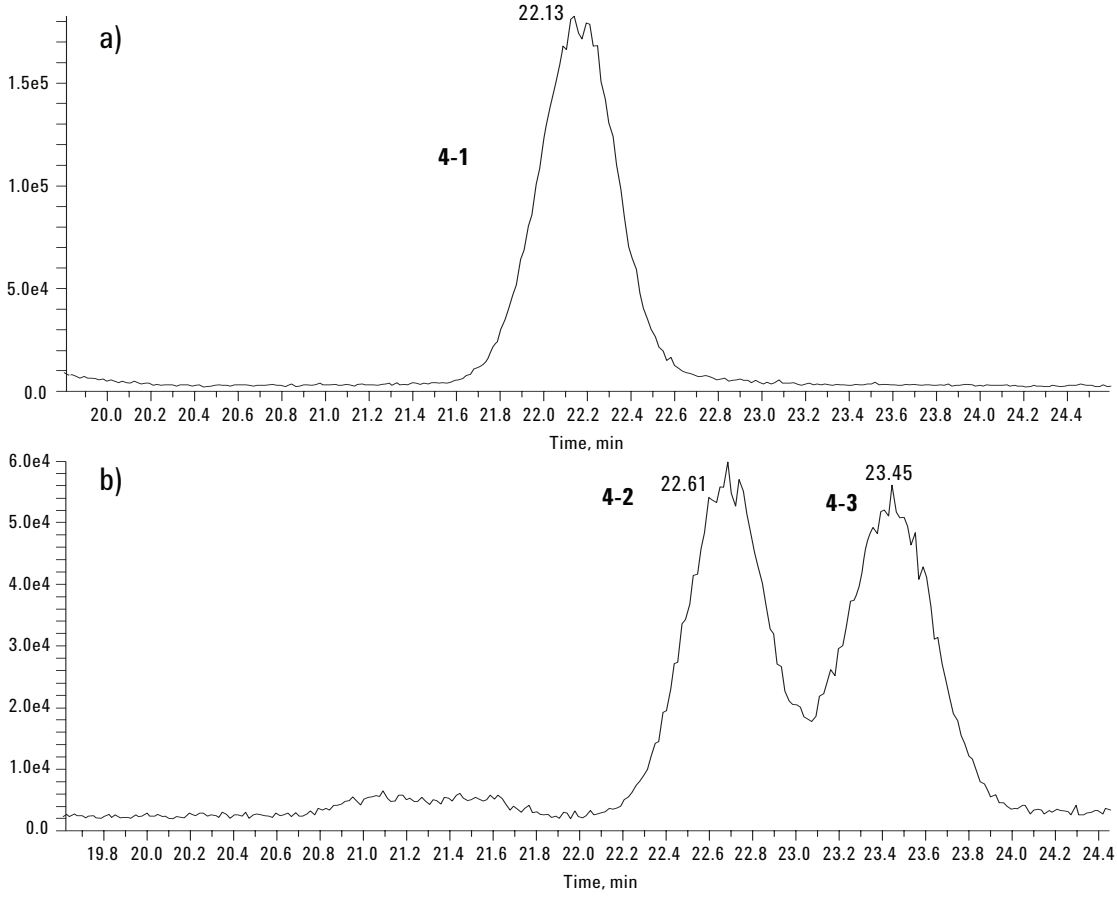

Figure 4. Extracted ion chromatograms for the three peaks of interest: a) *m/z* 436.98 - 437.48 and b) *m/z* 591.03 - 591.53.

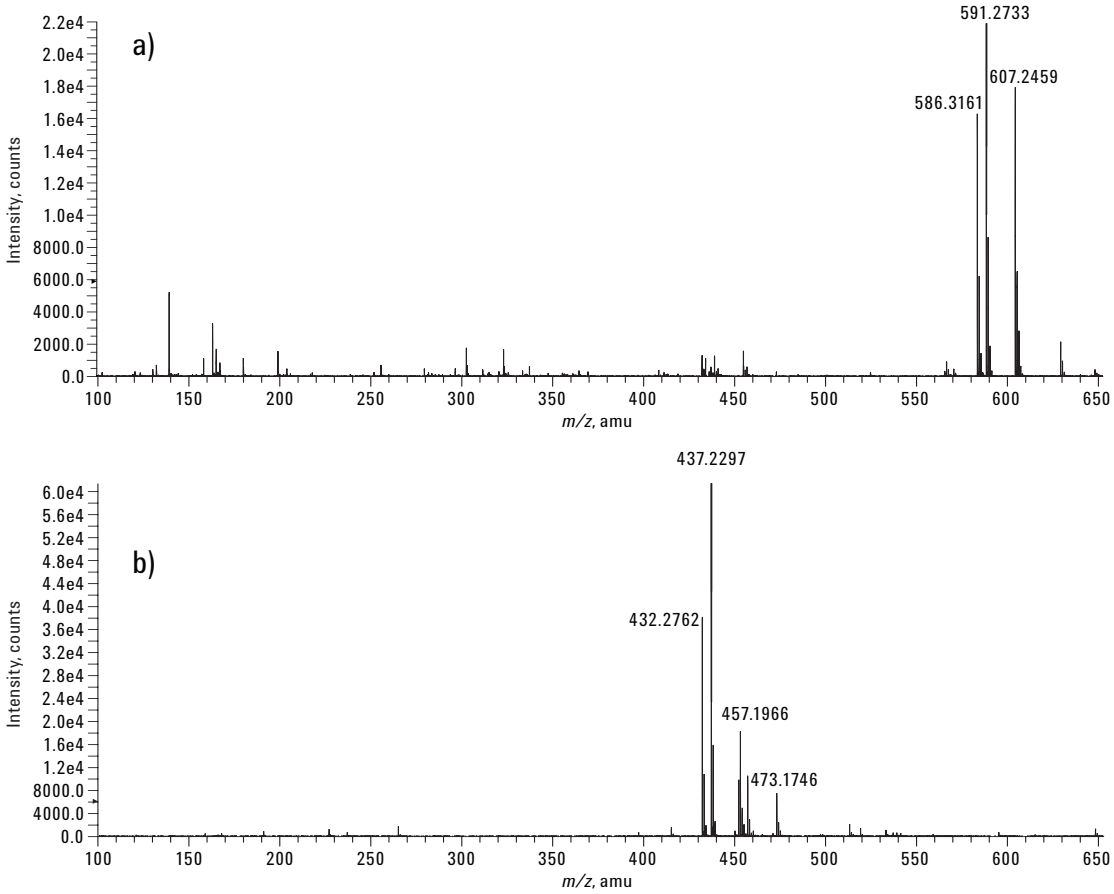

Figure 5. Mass spectrum of peak a) 4-1, b) 4-2, and 4-3.

Table 1. Molecular Formula Database Information for Peaks 4-1, 4-2 and 4-3

Peak	Mass	Molecular formula predicted	Theoretical mass	Mass error (PPM)	Molecular adduct
4-1	432.2746	$C_{25}H_{38}NO_5$	432.2744	0.12	$M+NH_4$
	437.2300	$C_{25}H_{34}O_5Na$	437.2298	0.35	M+Na
4-2	586.3163	$C_{36}H_{44}NO_6$	586.3163	–0.026	$M+NH_4$
	591.2722	$C_{36}H_{40}O_6Na$	591.2717	0.83	M+Na
4-3	586.3161	$C_{36}H_{44}NO_6$	586.3163	–0.37	$M+NH_4$
	591.2725	$C_{36}H_{40}O_6Na$	591.2717	1.3	M+Na

molecular formula consistent with that of BADGE.BuOH.HCl, as the HCl adds across the remaining epoxide ring. Figure 6 shows the relevant extracted ion chromatograms. Figure 7 shows the mass spectrum of BADGE.BuOH.HCl, with excellent correlation between experimental and theoretical chlorine isotope patterns.

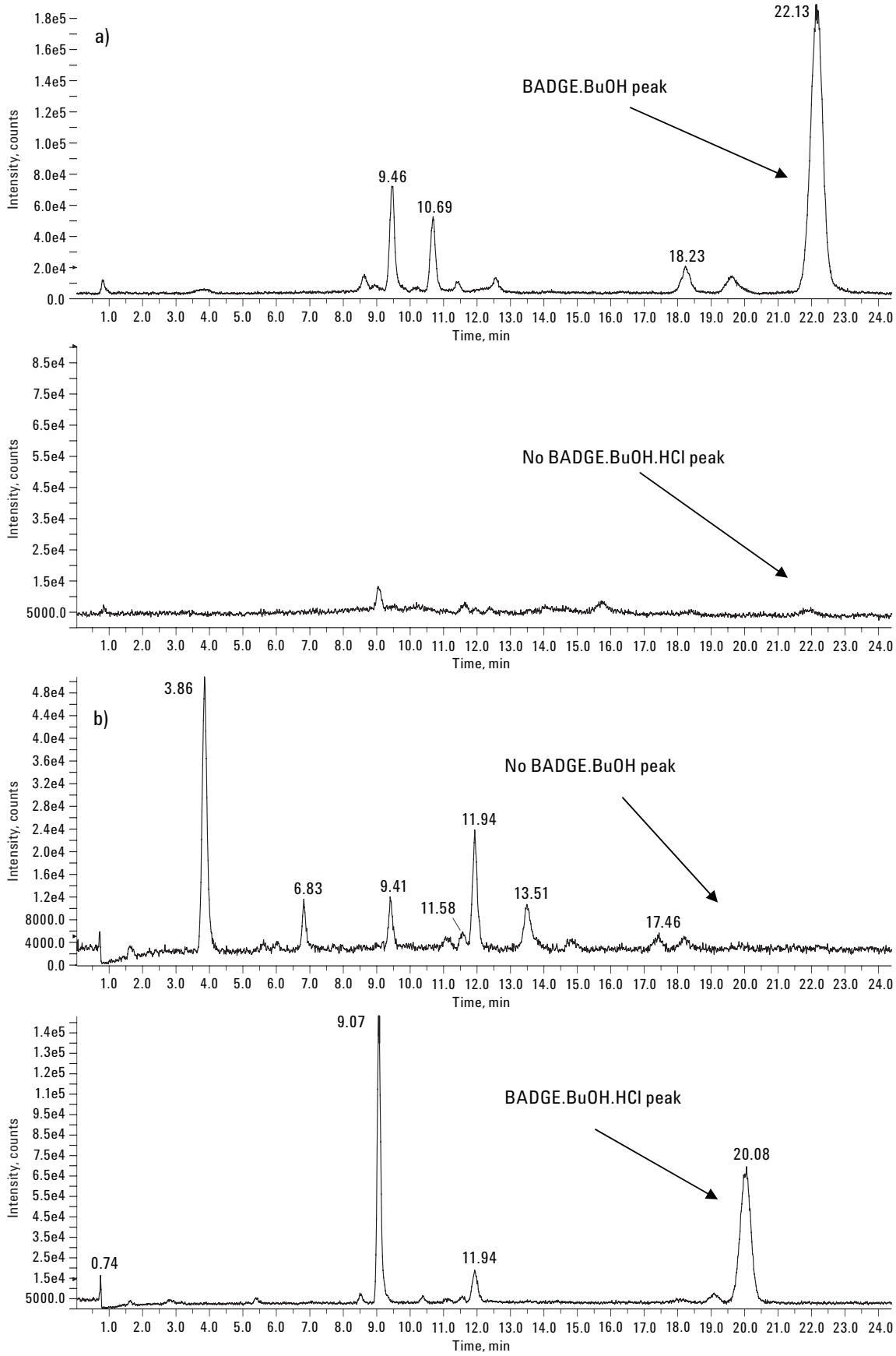

Figure 6. Extracted ion chromatograms for BADGE.BuOH (*m/z* 437 – 438) and BADGE.BuOH.HCl (*m/z* 451 – 452) for a) untreated EPH extract and b) EPH extract treated with HCl.

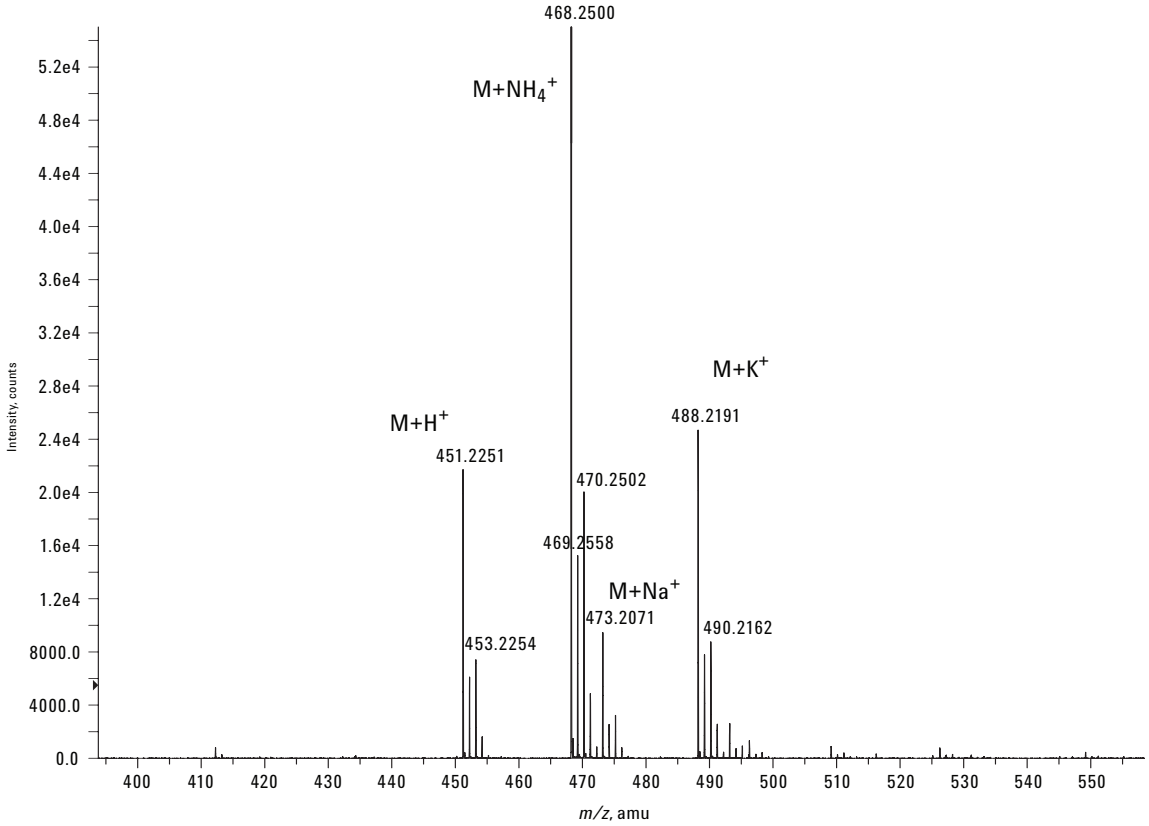

Figure 7. Mass spectrum of the peak at 20.1 min, corresponding to BADGE.BuOH.HCl.

Peaks 4-2 and 4-3 had the same mass spectra and were proposed to be $C_{36}H_{40}O_6$. As well as corresponding to cyclo-di-BADGE as suggested above, this could also correspond to BADGE.BPA, a linear BADGE derivative with the same molecular formula (see Figure 8).

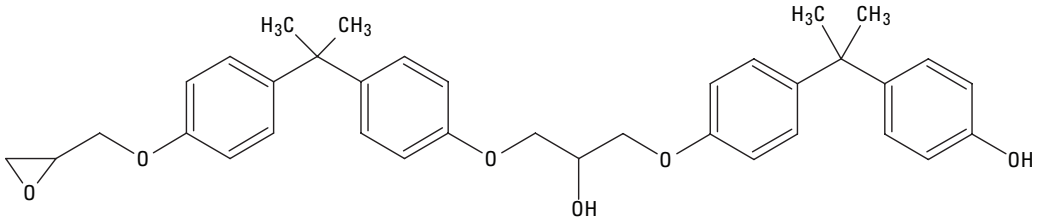

Figure 8. Structure of BADGE.BPA, $C_{36}H_{40}O_6$.

The addition of HCl did not change the two co-eluting peaks, which suggests that they are in fact due to cyclo-di-BADGE and not BADGE.BPA, as any peaks due to BADGE.BPA would be expected to disappear as the HCl will add across the epoxide ring. This conclusion was tested further; one of the differences between the proposed structures is that cyclo-di-BADGE has two hydroxide groups but BADGE.BPA has additional epoxide functionality as well as two hydroxide groups. It has been reported that acetic anhydride and TFAA react differentially with hydroxide and epoxide groups [3]. Acetic anhydride causes acylation of free hydroxide groups while further addition of TFAA causes acylation across the epoxide ring (see Figure 9). LC-TOF-MS analysis of the EPH extract after treatment with acetic anhydride showed the presence of a doubly acylated compound, either compound 9-1 or 9-2 ($C_{40}H_{44}O_8$). After further addition of TFAA the absence of a peak corresponding to compound 9-3 suggests that the two co-eluting peaks are in fact both due to cyclo-di-BADGE. The reason for two peaks is proposed to be because of the presence of stereoisomers, with the two hydroxide groups being cis- or trans- to each other, depending upon the side from which the phenol group attacks the epoxide ring during formation [3].

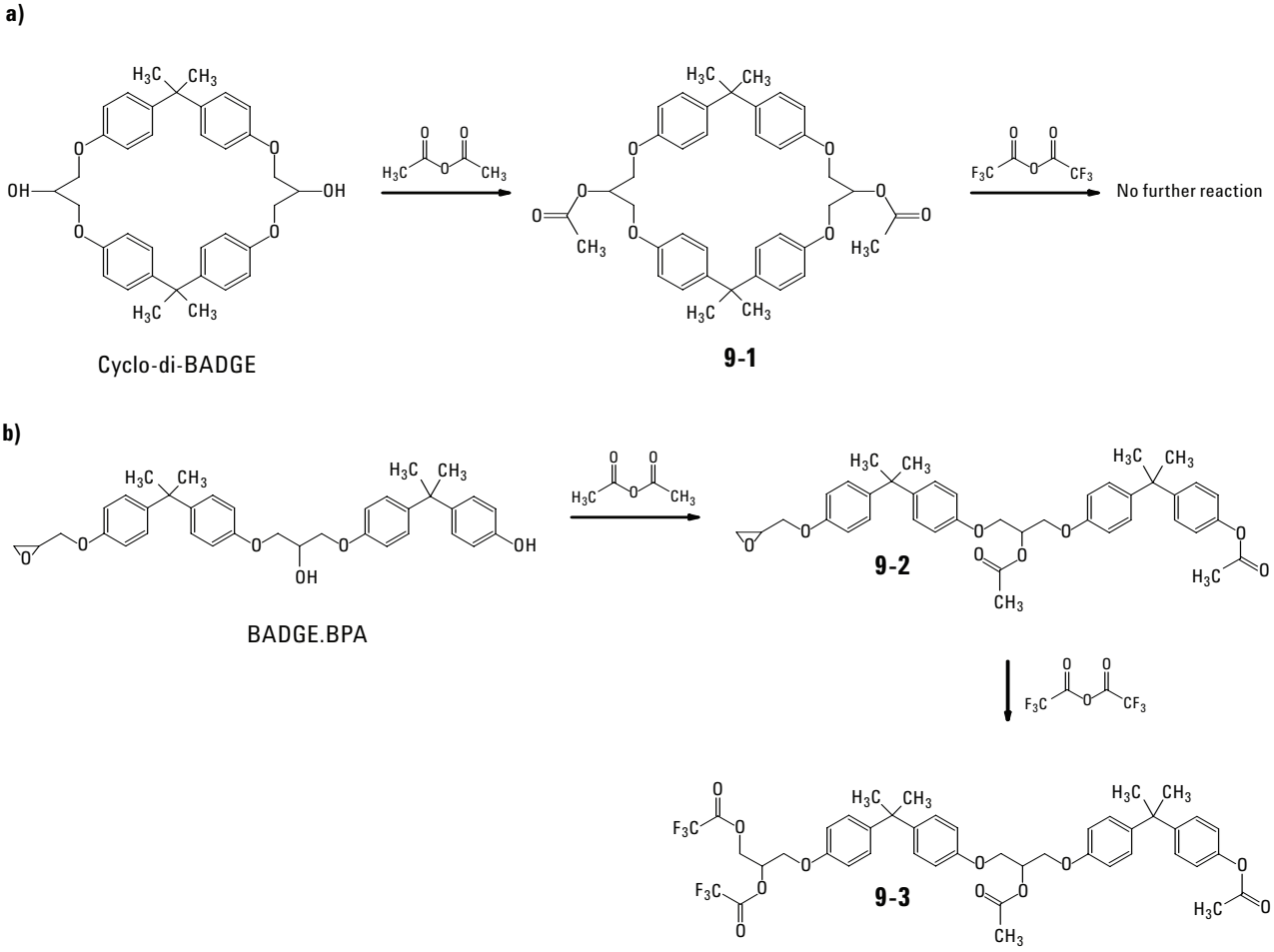

Figure 9. Acylation reactions for a) cyclo-di-BADGE and b) BADGE.BPA.

Conclusions

During migration studies at Leeds University, two co-eluting peaks were seen to behave differently during exposure to sunflower oil at different temperatures. There were in fact three co-eluting peaks and these were identified using LC-TOF-MS. The first peak was identified as BADGE.BuOH, and the second and third peaks were confirmed as *cis*- and *trans*- isomers of cyclo-di-BADGE. It is suggested that the differences seen in the original migration studies were due to the differences in structure between BADGE.BuOH and cyclo-di-BADGE, with the BADGE.BuOH migrating faster than cyclo-di-BADGE into the simulants, and the two stereoisomers of cyclo-di-BADGE migrating at the same rate as each other, which would account for the changes in peak height.

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