Excipient analysis by GPC/SEC and other LC techniques

Application compendium

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Agilent Technologies, Inc.

Verified for Agilent
1260 Infinity
GPC/SEC System

The Measure of Confidence

Agilent Technologies

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

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

Investigating excipients

Traditionally, excipients were inactive substances used in pharmaceutical and personal care formulations to carry active ingredients. At their most basic, excipients were employed to make a drug tablet or capsule large enough to be easily handled, because the active ingredient is present in small quantities. Thus a common painkiller may contain 80 percent or more inert filler. Other drug excipients ease the administration or uptake of active ingredients, or make them more palatable, or add color to aid identification. As well as these patient-friendly attributes, excipients can be used during manufacturing to assist handling of the active ingredient, for example by preventing it sticking to machinery or degrading during processing or storage. Many compound classes are used as excipients, including synthetic and natural polymers, saccharides, and proteins. However, similar compounds may have different functions in different formulations. Thus, carboxymethyl cellulose is used as a binder, a suspending agent and a disintegrant.

In the past, excipients were thought of as cheap and inert substances whose sole purpose was to carry active ingredients. However, it is now recognized that they can influence the rate and extent of uptake of actives. Moreover, there is a move away from synthetic excipients, for which it may be problematic to obtain regulatory approval, towards 'natural' compounds that are potentially less toxic, more easily accessible, cheaper, and more acceptable to consumers in this age of health scares associated with synthetic products.

The value of excipients has therefore led to extensive research by the pharmaceutical industry to improve their efficacy. This list shows some excipients released since 2006[1].

- Modified excipients - Polyplasdone Ultra (ISP), Lμtrol micro 68 & 127; Kollidon CL-F & CL-SF (all BASF), Swelstar MX 1 (Asahi Kasei), GalenIQ 721 (Palatinit)
- Co-processed excipients - Spectrablend HS (Sensient), Prosolv ODT (JRS), Ludiflash (BASF), Aquarius (Ashland), Avicel DG (FMC), Sepitrap (Seppic), Starcap 1500 (Colorcon)
- Novel excipients - Solutol HS 15, Soluplus, Kollicoat Smartseal 30 D (all BASF)

The exact nature and formulation of many excipients are considered trade secrets. However, commercial confidentiality has quality implications for drug companies when using these compounds, particularly because of new regulatory requirements. For example, under ICH M4Q (Quality)[2], novel excipients now require characterization of their functionality (pharmaceutical assessment and drug delivery properties) and physicochemistry (physicochemical properties and impurities).

[1] Trademarks are the property of their respective owners.

[2] The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Quality - M4Q(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Geneva, Switzerland.

Quality by design for pharma

The Quality by Design (QbD) concept was developed by JM Juran over the past 20 years. Juran's approach focused on planning for quality as part of the manufacturing process, i.e. establishing processes at the outset in such a way that quality problems were designed out. In effect, product development was based on quality risk management. QbD was taken up by the US Food and Drug Administration in 2002[3] and continues to be refined. The ongoing need for QbD in the pharmaceutical industry is suggested by a 300 percent increase in pharma product recalls from 2008 to 2009. Some of these recalls may have been caused by problems with excipients. However, manufacturers of excipients may only provide material safety data sheets to end users, with no information on characterization. This obviously has implications for pharma companies using QbD because quality requires a good understanding of the characteristics of all the components of a medicine.

Agilent has a long history of involvement in the analysis of excipients by gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC). Gel permeation chromatography is an important technique for assessing the molecular weight distribution of excipient polymers, because molecular weight influences many of their physical characteristics. Thus a molecular weight distribution higher or lower than required suggests that the polymer will not behave as required in end use.

This application booklet includes a wide range of applications that illustrate the performance of Agilent solutions for excipient analysis. Although the main focus is on GPC/SEC, other applications highlight HPLC, gradient polymer elution chromatography and liquid chromatography under critical conditions.

[3] Pharmaceutical Quality for the 21st Century - A Risk-Based Approach. USFDA.

Binders

Excipient binders hold tablets together. They add mechanical strength so that tablets do not disintegrate during manufacture, transport, storage or handling. Binders also add bulk to low doses of active ingredient.

Polyvinylpyrrolidone

Excipient polyvinylpyrrolidone (PVP) has has been used in more than one hundred drugs. It is available in three forms; soluble povidone, insoluble crosslinked crospovidone, and copovidone, a water-soluble copolymer of vinylpyrrolidone and vinyl acetate. Although povidone is mainly used as a tablet binder, it is also employed as a dissolving and flow assistant, dispersant, and stabilizer for heat-sensitive actives. Crospovidone is a solubilizer with superdisintegrant properties, used for dispersing solid oral pharmaceuticals after ingestion to increase the rate of absorption. Copovidone is also a tablet binder, used for wet and dry granulation.

Povidone is soluble in polar organics and water. In this example, dimethyl formamide (DMF) is used with lithium bromide to minimize any polyelectrolyte effects. The response in DMF is relatively small using an RI detector. Polyvinyl pyrrolidone can be chromatographed successfully with Agilent PLgel 10 µm MIXED-B columns (Figure 1).

Columns: 2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent: DMF + 0.1% LiBr
Flow Rate: 1.0 mL/min
Temp: 70 °C
System: Agilent 1260 Infinity GPC/SEC Analysis System (RI)

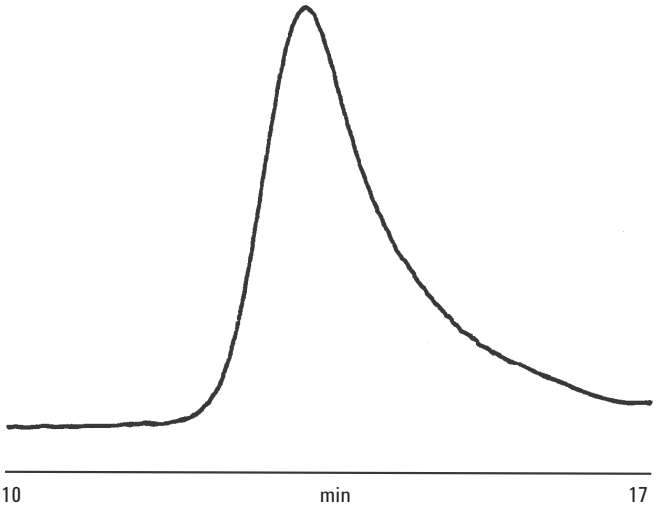

Figure 1. *Analysis of polyvinylpyrrolidone using Agilent PLgel 10 µm MIXED-B columns*

Polyvinylpyrrolidone is also used in personal care products. In this example, PVP was a constituent of a soap formulation, soluble in the polar eluent, dimethylacetamide (DMAc). It was successfully analyzed using PLgel 10 µm MIXED-B columns (Figure 2). A broad peak was obtained, typical of a polydisperse sample.

Columns: 3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent: DMAc + 0.5 % LiBr
Flow Rate: 1.0 mL/min
Loading: 0.2 % w/v, 100 µL
Temp: 60 °C
System: 1260 Infinity GPC/SEC Analysis System (RI)

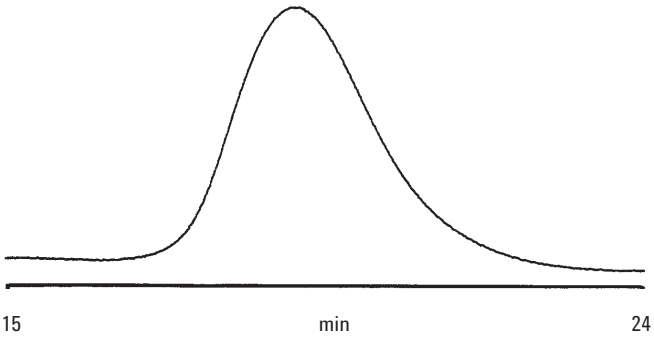

Figure 2. *Polyvinylpyrrolidone on an Agilent PLgel 10 µm MIXED-B three-column set*

As mentioned on page 5, polyvinylpyrrolidone is soluble in aqueous solvents as well as polar organics. Figure 3 shows the distribution of a broad distribution sample, as expected with this type of material.

Columns: Agilent PL aquagel-OH 60 8 µm, 300 x 7.5 mm
(Part No. PL1149-6860)
PL aquagel-OH 50 8 µm, 300 x 7.5 mm
(Part No. PL1149-6850)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 3
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: Agilent PL-GPC 50 Integrated GPC/SEC System (RI)

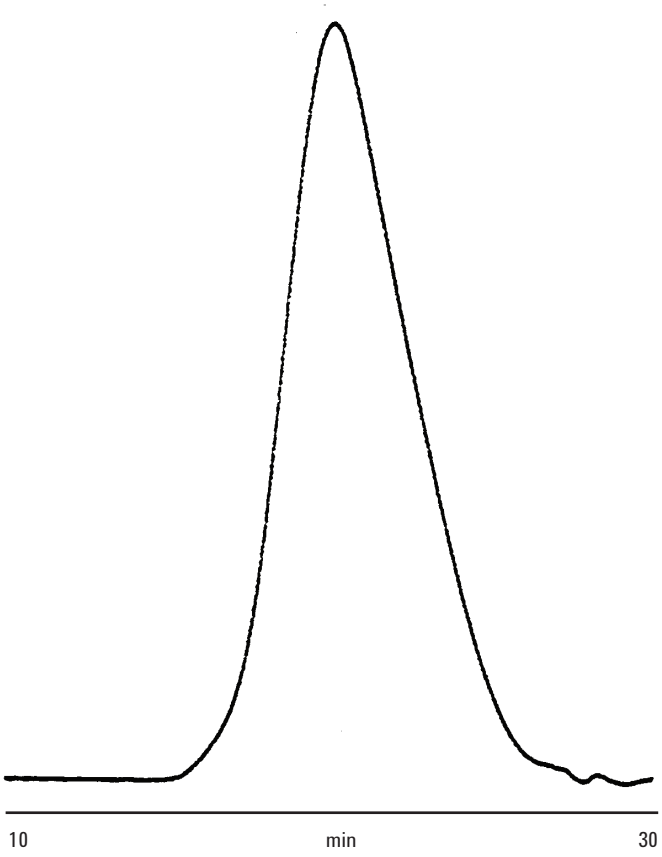

Figure 3. *Raw data chromatogram of a broad-distribution polyvinyl pyrrolidone in an aqueous solvent with Agilent PL aquagel-OH columns*

The Agilent PL-GPC 50 Integrated GPC/SEC System

Polyethylene glycol - *by liquid chromatography under critical conditions*

Polyethylene glycol is widely used in pharmaceutical and consumer-care products. Lower-molecular-weight types are employed as solvents in liquids and soft capsules. Solid PEGS are used as ointment bases, binders, film coatings, and lubricants.

Liquid chromatography under critical conditions (LCCC), or critical point chromatography, is a technique used to investigate very small differences between the chemical structures of polymers such as PEGs. These differences could arise through the use of co-monomers or through the introduction of end-group functionality. Traditional interactive chromatographic techniques are often insensitive to small changes in structure and critical point chromatography has become the method of choice for these analyses.

In gel permeation chromatography, large molecules elute before small molecules due to the exclusion from the porous packing material. Conversely, in interactive chromatography, large molecules elute after small molecules as they interact with the packing material in the column to a greater extent. The critical point is defined as the eluent conditions that promote a balance between SEC and interactive mechanisms such that molecules elute at the same retention time regardless of MW. At the critical point small changes in chemistry such as type of end-group can cause big changes in elution behavior.

LCCC is useful for the analysis of polyethylene glycol that had been modified with amine end groups. The structure of original and modified PEG materials is shown in Figure 4.

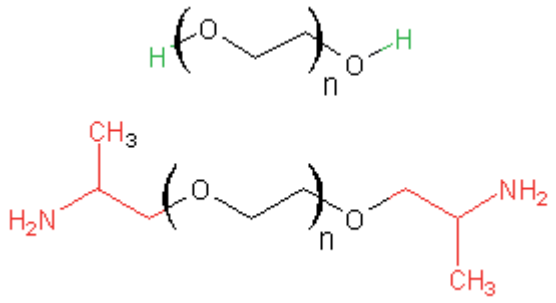

Figure 4. *Structure of original (upper) and modified (lower) polyethylene glycol*

Critical point conditions for PEG were established by analyzing a series of PEG narrow standards of different molecular weights using different isocratic combinations of acetonitrile and water. The analysis of PEG by GPC/SEC is so well understood that it is commonly employed as a standard for calibrating the columns used in these techniques. Figure 5 shows chromatograms of the standards in SEC and reversed phase mode, and at the critical point where elution is independent of molecular weight.

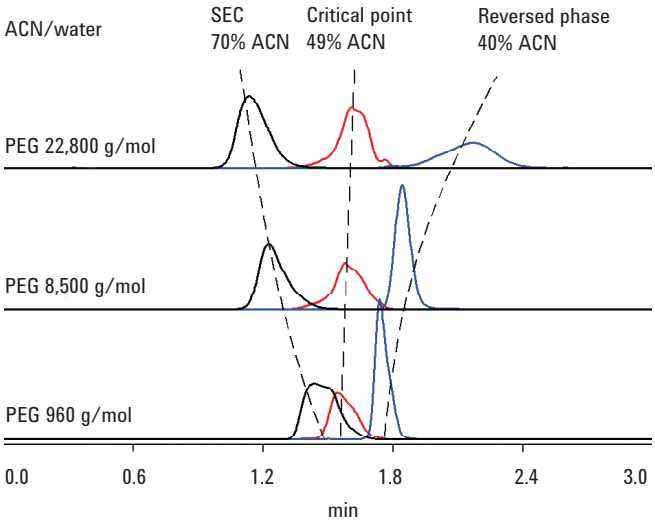

Figure 5. *Polyethylene glycol analyzed by SEC and reversed phase to reveal the critical point*

Figure 6 shows a chromatogram of the amine-modified PEG material, before and after neutralization of the amine functionality with hydrochloric acid.

Column: Agilent PLRP-S 100Å 5 µm, 150 x 4.6 mm (Part No. PL111-3500)
Eluent: 49% Acetonitrile in water
Flow Rate: 1.0 mL/min
Inj Vol: 20 µL
Detection: Agilent 380-LC ELSD

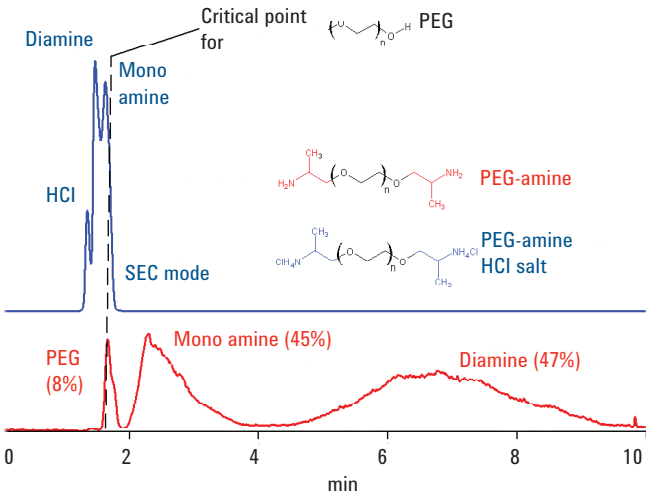

Figure 6. *Amine-modified polyethylene glycol before (lower) and after (upper) neutralization with hydrochloric acid*

Before adding the acid, one peak was observed at total permeation (corresponding to unmodified PEG) and two peaks were observed eluting in interactive mode (after total permeation of the column). The two peaks eluting in interactive mode were assigned as the mono- and diamine end-group-modified PEGs. Based on the peak areas, the ratio of components was assigned as 8% PEG, 45% monoamine and 47% diamine. Adding hydrochloric acid changed the elution to SEC mode (elution before the PEG peak), indicating the sensitivity of the chromatography to sample chemistry at critical conditions.

The Agilent 385-LC and 380-LC evaporative light scattering detectors

Pectin

Pectin is a natural product used for coating capsules. 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. Size exclusion chromatography 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 MIXED-H 8 µm are the columns of choice for this application.

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 7.

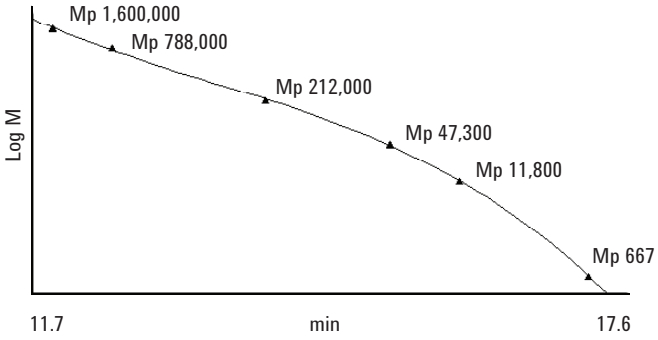

Figure 7. *Pullulan standard calibration curve for the Agilent PL aquagel-OH MIXED-H 8 µm column*

Raw-data chromatograms for the pectin samples are illustrated in Figure 8.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

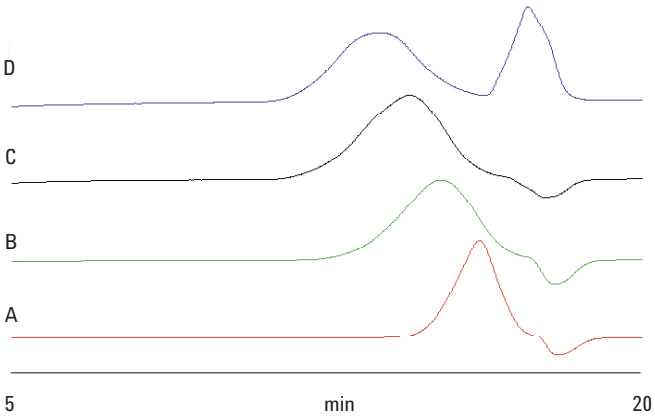

Figure 8. *Chromatograms of four pectin samples on Agilent PL aquagel-OH MIXED columns*

Overlaid molecular weight distribution plots are shown in Figure 9.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2 M NaNO3 + 0.01 M NaH2PO4 at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

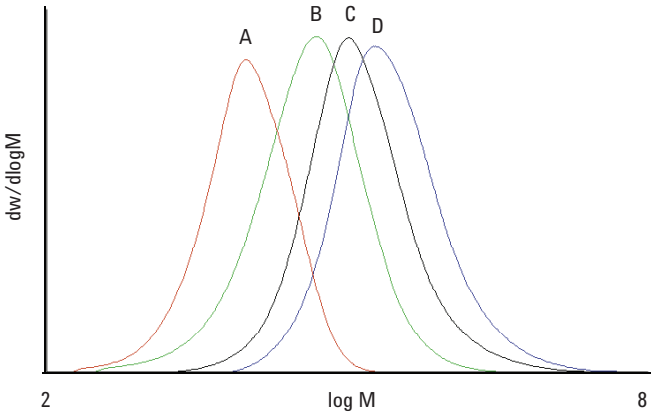

Figure 9. *Molecular weight distributions of four pectins*

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 Table 1.

Table 1. *Molecular weight averages and polydispersity for four pectin samples*

Sample	Molecular weight average		Polydispersity (Mw/Mn)
	Mn	Mw	
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

The samples vary in molecular weight and in polydispersity (Mw/Mn).

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.

Chitosan

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 just to a family of copolymers with various fractions of acetylated units containing 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 that are usually neutral or negatively charged. Pharmaceutical applications of chitosan include biomedical (e.g. wound healing, burn treatment and use as a hemostatic agent), tablet compression, disintegration, and dissolution, and as a controlled release agent.

GPC/SEC can be used as a quality control tool for the determination of Mw and molecular weight distribution of chitosan, and so three grades of chitosan were analyzed using a column set comprising 2 x Agilent PL aquagel-OH MIXED-H 8 µm columns. Due to the cationic nature of the samples, they were prepared in strong acid and allowed to stand overnight to aid dissolution. The samples were then analyzed in 0.5 M sodium nitrate buffer and at low pH. The system was calibrated with narrow pullulan polysaccharide standards, also from Agilent Technologies.

An example calibration curve for the PL aquagel-OH MIXED 8 µm columns using pullulan standards is shown in Figure 7 (page 9).

Raw-data chromatograms and weight average molecular weight values (Mw) for the three chitosan samples are shown in Figure 10. Marked imbalance peaks (unequal positive and negative peaks) were observed on the RI due to the fact that the samples were prepared in strong acid for dissolution.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.5 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 2
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

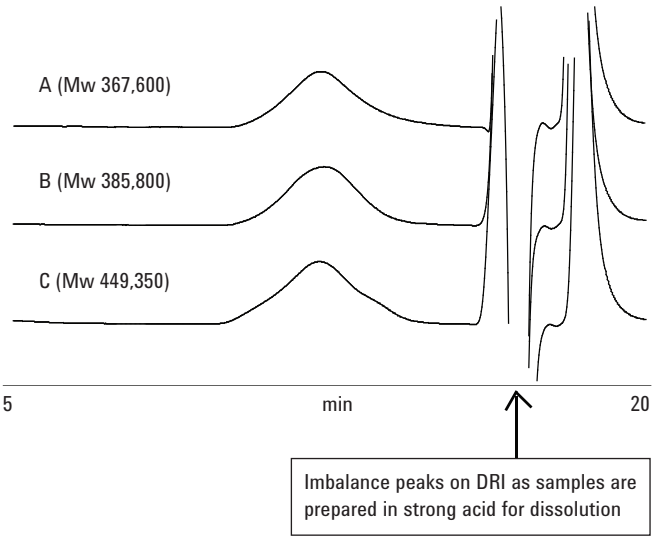

Figure 10. *Raw-data chromatograms and molecular weight averages of three chitosan samples*

The subtle differences in molecular weight revealed in this analysis are sufficient to change the behavior of the three chitosan samples in end-use application.

Methyl cellulose

This polymer is a cellulose derivative used as a dry binder, added during direct powder compression or after wet granulation. Methyl cellulose is also employed as a thickener and emulsifier in cosmetics, and as a treatment for constipation. Two samples of methyl cellulose were analyzed by SEC using PL aquagel-OH columns. The calculated molecular weight averages were compared with manufacturers' viscosity values. Calibration was done using Agilent pullulan polysaccharide standards. Figure 11 shows the raw-data chromatograms for the two methyl celluloses. A good correlation between viscosity and molecular weight averages was obtained, as shown in Table 2.

Table 2. *Viscosity and molecular weight ranges of two samples of methyl cellulose*

	Methyl cellulose sample	
	A	B
Viscosity range (cps)	85 to 115	4,000 to 6,000
Mn	131,000	484,000
Mw	369,000	1,023,000
Mz	691,000	1,884,000

Columns: PL aquagel-OH 60 8 µm, 300 x 7.5 mm (Part No. PL1149-6860)
PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent: 0.05 M NaH_2PO_4 + 0.25 M NaCl at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

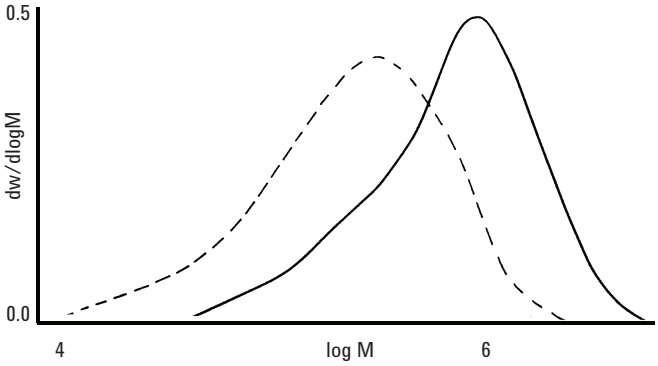

Figure 11. *Raw-data chromatograms for two methyl celluloses on an Agilent PL aquagel-OH column set*

Coatings

Solid-dose pharmaceuticals are often coated with thin layers of excipient to protect the drug, prevent premature disintegration of the tablet, modify release of the active ingredient, or even to provide a substrate for product labelling or identification. Gelatin is one of the oldest and most commonly used coatings.

Gelatin

Size exclusion chromatography of gelatin yields critical molecular weight information upon which the physical properties of the polymer (such as the setting properties) depend. Linear PL aquagel-OH MIXED-H 8 µm columns were used in this investigation. The use of a simple buffer solution as the eluent for the analysis of gelatins reduces interaction between the sample and the columns, ensuring that good chromatography is obtained.

Light scattering was used for detection because the technique provides absolute molecular weight without the need for column calibration.

The eluent was prepared as a buffer with its pH adjusted by the addition of 0.1 M NaOH. The sample was accurately prepared as 1.0 mg/mL solutions in the eluent. The light scattering detector was first calibrated using an Agilent pullulan polysaccharide standard. The standard was Mp 186,000, prepared at 1.0 mg/mL. From the known concentration, Mp and dn/dc of the calibrant, the detector constants and inter-detector volume for the system were calculated.

From the refractive index chromatogram, dn/dc could be calculated as the gelatin sample was prepared at known concentration. This value of dn/dc was then used to calculate a bulk Mw value from the 90° to the 15° light scattering data.

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

Figure 12 shows the RI and the 90° and 15° light scattering data for the gelatin.

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

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm
(Part No. PL1149-6800)
Eluent: Water + 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
System: PL-GPC 50 Integrated GPC/SEC System
(RI and Agilent PL-GPC 50 Dual Angle Light Scattering Detector)

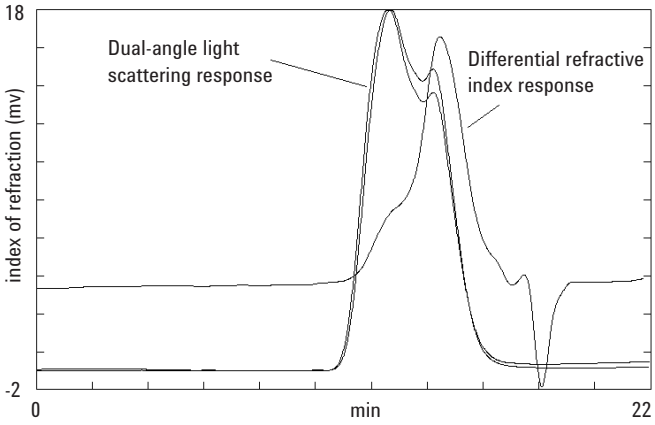

Figure 12. *Refractive index, and 90° and 15° light scattering data for gelatin, on an Agilent PL aquagel-OH MIXED-H two-column set*

Cellulose acetate

Cellulose is esterified with acetyl, butyryl or phthalate radicals to form a mixed ester that is used as a slow-release coating for capsules and tablets. 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.

Columns: 3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm
(Part No. PL1110-6100)
Eluent: DMAc + 0.5 % LiBr
Flow Rate: 1.0 mL/min
Loading: 0.2 % w/v, 100 µL
Temp: 60 °C
System: 1260 Infinity GPC/SEC Analysis System (RI)

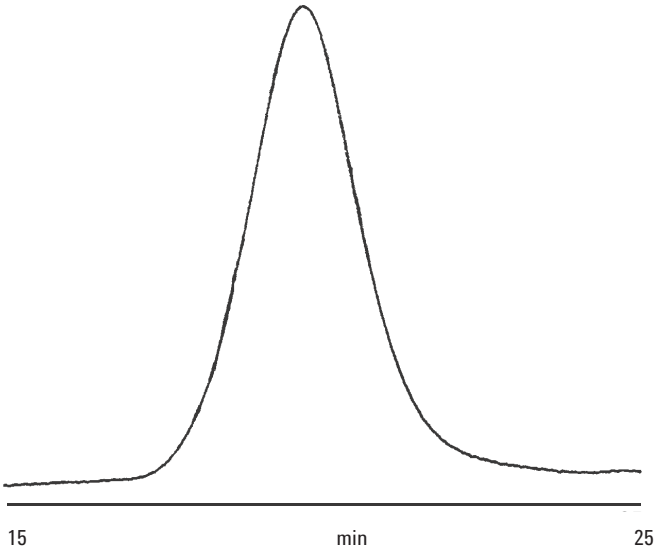

Figure 13. *Analysis of cellulose acetate using Agilent PLgel 10 µm MIXED-B columns*

The Agilent 1260 Infinity GPC/SEC Analysis System

Disintegrants

Disintegrants such as carboxymethyl cellulose expand and dissolve when wet, causing tablets to break apart and release the active ingredient. Disintegrants can be modified, or different disintegrants combined, so that the tablet dissolves over a period of time to deliver slow release of the active ingredient.

Carboxymethyl cellulose

Size exclusion chromatography can be used to reveal slight differences in the molecular size profiles of water soluble polymers that are within the same viscosity grade. Polymers such as carboxymethyl cellulose (CMC) may have different physical characteristics due to the variation in the molecular weight of the material. Agilent PL aquagel-OH 40 8 µm and PL aquagel-OH 60 8 µm columns are ideal for distinguishing fine variations in CMC molecular weights, 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 versions of PL aquagel-OH were connected in series to cover a molecular weight range from 10^4 to 10^7. Column calibration was achieved using pullulan standards, also from Agilent.

Figure 14 shows the slight differences in molecular weights of three carboxymethyl celluloses that lie within the same viscosity range.

Columns: 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: 0.5 M Na_2SO_4
Flow Rate: 1.0 mL/min
System: PL-GPC 50 Integrated GPC/SEC System (RI)

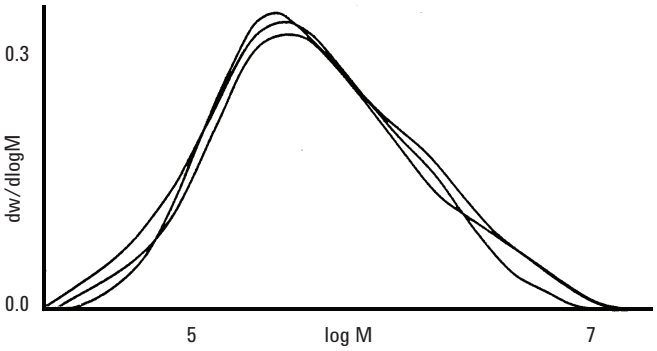

Figure 14. *Raw-data chromatograms produced by Agilent PL aquagel-OH columns, showing slight differences in the Mw of three carboxymethyl celluloses lying within the same viscosity range*

Cyclodextrins - *by reversed phase HPLC*

Cyclodextrins (CDs) are cyclic oligosaccharides, typically composed of 6, 7 or 8 glucose units, or α-, β- or γ-cyclodextrin, respectively. Their shape resembles a shortened cone with a relatively hydrophobic cavity and a hydrophilic exterior. The structure of cyclodextrins allows them to interact with appropriately sized molecules to form inclusion complexes. Such behavior has been recognized by the pharmaceutical industry, where CDs are used in drug delivery to modify the physiochemical properties of the target drug.

Cyclodextrins are commonly used with hydrophobic drug molecules to improve the target compound's solubility, stability, bioavailability and dissolution. The type of cyclodextrin determines the extent of modification to the molecule's properties. In addition, the choice of the cyclodextrin used will depend on the requirements of the dosage form, and route of delivery, as well as the solubilizing capacity needed to carry the drug load. The use of cyclodextrins to facilitate the dissolution of hydrophobic drugs provides a drug delivery system capable of enabling the development of drugs otherwise discounted from development due to their insolubility. Consequently, their characterization is of great importance within the pharmaceutical sector.

HPLC analysis of cyclodextrins is difficult because they do not possess a UV chromophore, and gradient elution is often required, making RI detection impractical. Evaporative light scattering detection (ELSD) provides a beneficial alternative for the determination of cyclodextrins because it is not dependent on a compound's optical properties. For this reason ELSD is often referred to as a 'universal' detector.

The ability of Agilent ELSD to detect any compound that is less volatile than the mobile phase facilitates the detection of components of pharmaceutical formulations in a single chromatogram. The Agilent ELSD is also capable of operating at the low temperatures typically required for pharmaceutical compounds, because its operating temperature is independent of the mobile phase.

The benefits of ELS detection are highlighted in Figure 15 for the analysis of a pharmaceutical formulation containing cyclodextrins and an active drug compound, ibuprofen.

Column: C18 5 µm, 150 x 4.6 mm
Eluent A: Water
Eluent B: Acetonitrile
Flow Rate: 1.0 mL/min
Inj Vol: 20 µL
Gradient: 50 to 95% B in 5 min
Detection: Agilent 380-LC ELSD (neb=30 °C, evap=50 °C, gas=1.0 SLM)

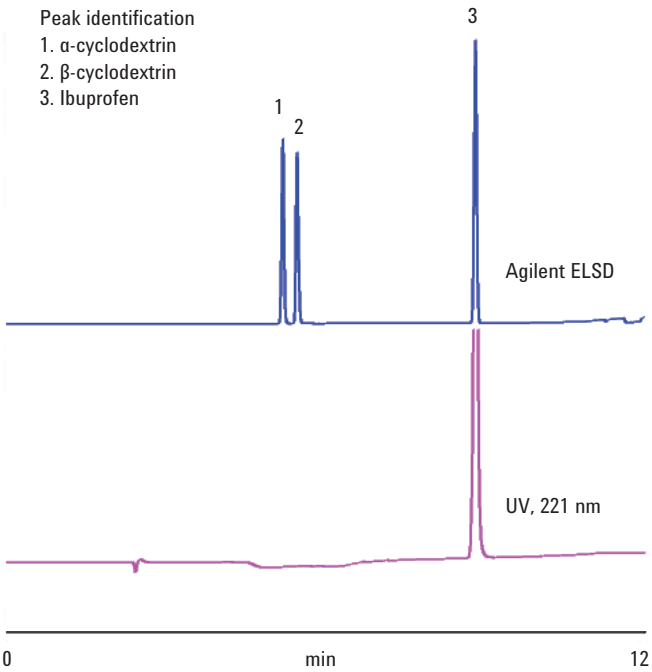

Figure 15. *The presence of cyclodextrins revealed by Agilent ELS detection*

The low temperature advantage of the Agilent ELSD is demonstrated in Figure 16 with the same formulation. Operating the instrument at 30 °C provides the true composition of the sample mixture. However, at a temperature of 50 °C, loss of the semi-volatile ibuprofen occurs, which underestimates the concentration of the active drug. Therefore, the Agilent ELSD can provide the true composition of pharmaceutical formulations in a single chromatogram.

Column: C18 5 µm, 150 x 4.6 mm
Eluent A: Water
Eluent B: Acetonitrile
Flow Rate: 1.0 mL/min
Inj Vol: 20 µL
Gradient: 50 to 95% B in 5 min
Detection: Agilent 380-LC ELSD (neb=30 °C, evap=50 °C, gas=1.0 SLM)

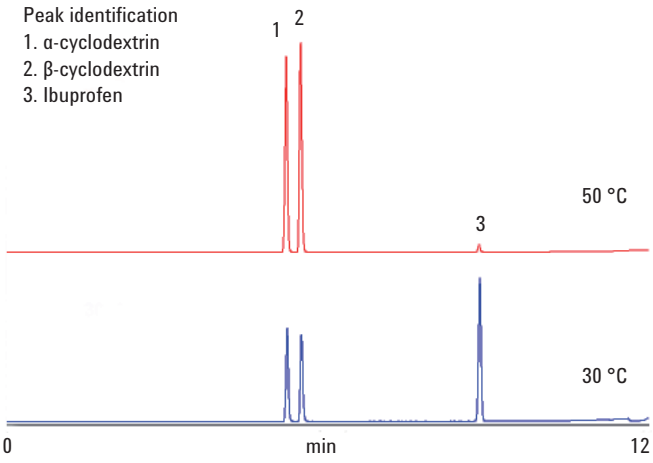

Figure 16. *Low temperature operation of the Agilent ELSD shows the true concentration of a pharmaceutical formulation*

The Agilent ELSD reveals the true composition of cyclodextrin and ibuprofen due to its sensitivity to compounds that possess weak or no UV chromophores. The instrument surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications.

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 and potentially ineffective. 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. Controlled delivery can be provided by excipients.

Polycaprolactam

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. The chromatogram in Figure 17 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.

A large set of positive and negative system peaks was observed after the broad polymer peak. This is common with refractive index detectors and is an artifact.

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
System: PL-GPC 50 Integrated GPC/SEC System (RI)

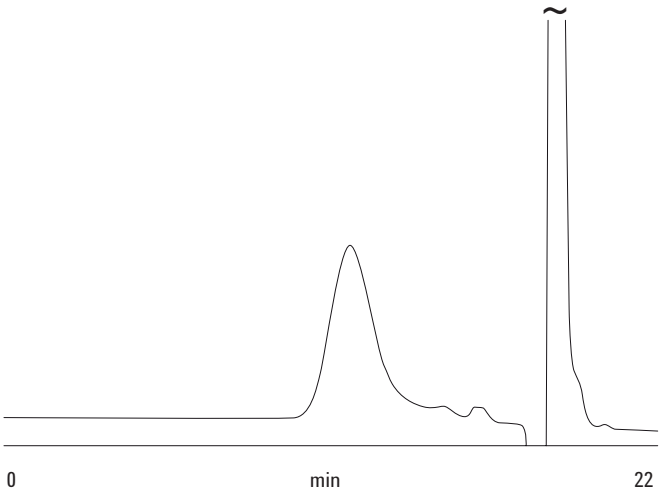

Figure 17. *The broad molecular weight distribution of a polycaprolactam revealed by gel permeation chromatography with Agilent PLgel MIXED-C columns (column peak has been cut)*

Poly(lactide-*co*-glycolide) - *by gradient polymer elution chromatography*

Polylactide and its copolymers with glycolide were originally designed for biodegradable sutures. However, they found wider use as components of drug delivery systems because of their ability to modify the pharmacokinetics of the active ingredient and protect them from degradation in the body.

This example describes the compositional analysis of a random copolymer of lactide and glycolide using gradient polymer elution chromatography (GPEC). GPEC is a generic term that describes the analysis of polymers under chromatographic conditions where a gradient is applied between two solvents. A GPEC analysis can be used to separate a series of polymers on the basis of molecular weight, but is more usually applied to separate polymers on the basis of chemical composition. Typically, distributions in chemical composition result from heterogeneities in the polymerization reaction or from the copolymerization of monomers to form random or block copolymers.

The equipment used in a GPEC experiment is identical to that used in traditional gradient LC techniques; a binary gradient pump, an injection valve, a reversed or normal phase HPLC column (although GPEC has been performed on totally inert substrates) and a detector insensitive to eluent composition, typically, an evaporative light scattering detector. The gradient used in the analysis is typically a binary system consisting of a thermodynamically good solvent and a thermodynamically poor solvent for the polymer. Initially, the thermodynamically poor solvent is introduced to the system. A sample of polymer under investigation is dissolved in the good solvent and injected onto the column, and a gradient is then initiated which moves from the poor solvent to the thermodynamically good solvent for the polymer. Poor solvent conditions prevail at the start of the analysis and the polymer precipitates from solution. As the gradient progresses, thermodynamically favorable conditions are achieved resulting in the polymer re-dissolving. When the polymer is in solution, interactions with the surface of the media in the column can occur, the mechanism of the interaction dependent on the column and eluent selected. These interactions are dominated by either a reversed/normal phase mechanism or a size exclusion mechanism that results in retention or acceleration of the polymer relative to the solvent front, respectively. The nature of the interactions with the column determines which parameters control the separation. If the polymer is retained on the column relative to the solvent front, the polymer remains in the zone of good solubility and the separation is controlled by molecular weight (as in a typical reversed or normal phase HPLC experiment). However, if the polymer is accelerated relative to the good solvent front, the sample enters the zone of poor solubility and re-precipitation occurs. The solvent front continues down the column and so good solvent conditions are re-established and the polymer re-dissolves. Under these conditions, this process occurs many times and successive precipitation/dissolution steps continue until the polymer elutes from the column. Elution of the sample is controlled by the relative solubility of polymer components in the two eluents and so a compositional separation is obtained.

Figure 18 shows a generic structure for the copolymers. For this analysis, tetrahydrofuran was selected as the thermodynamically good solvent while methanol was selected as the poor solvent.

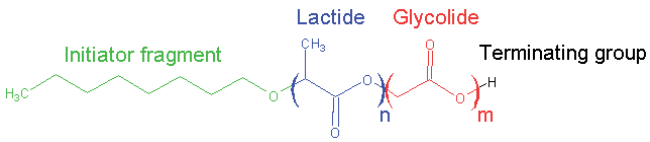

Figure 18. *Structure of poly(lactide-co-glycolide) random copolymers*

Figure 19 shows chromatograms of the copolymers obtained using a gradient consisting of a 2 minute hold in 99% methanol followed by 1% to 99% tetrahydrofuran in 10 minutes.

Column:	Agilent PLRP-S 100Å 5 µm, 150 x 4.6 mm (Part No. PL111-3500)
Eluent:	THF and methanol
Flow Rate:	1.0 mL/min
Inj Vol:	20 µL
Temp:	Ambient
Detector:	Agilent 380-LC ELSD
System:	1260 Infinity Modules

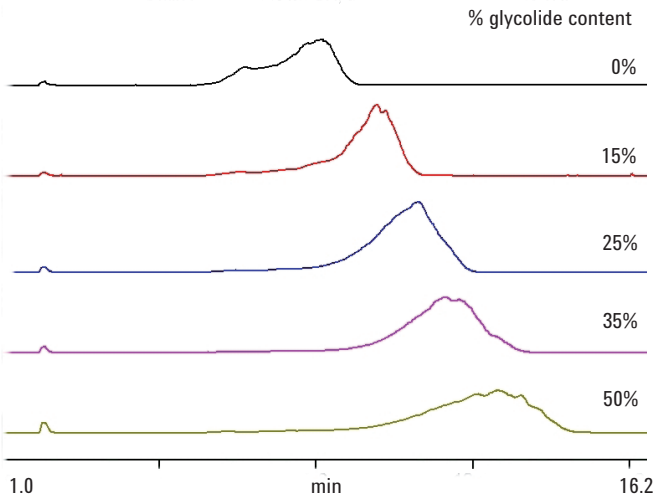

Figure 19. *Chromatograms of poly(lactide-co-glycolide) copolymers at different concentrations of tetrahydrofuran*

Figure 20 displays a correlation of the percent glycolide content and the molecular weight of the samples as a function of the observed retention time. Clearly, there was no correlation between the molecular weight of the copolymers and the retention times. However, a linear relationship was apparent between percent glycolide in the copolymers and the retention time, demonstrating that a compositional separation had been obtained.

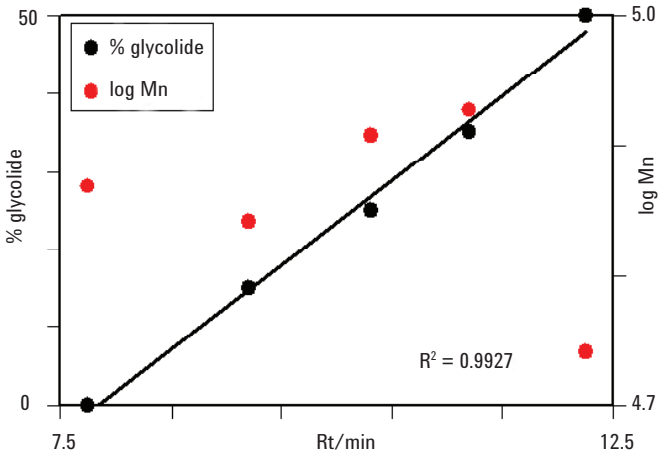

Figure 20. *Correlation of percent glycolide content and the molecular weight of the samples as a function of the observed retention time*

GPEC is thus a powerful analytical tool for probing the molecular structure of polymers, as composition and molecular weight sensitive separations can be performed by careful selection of eluents and columns. The disadvantage of this sensitivity to the analysis conditions is that to get the most from a GPEC experiment, the analysis conditions must be specifically designed to suit individual applications.

Fillers

To make an oral solid-dose tablet or capsule practical to manufacture and convenient to take, it needs to be of a suitable size, i.e. from a few millimeters to about a centimeter. Fillers such as sugar alcohols are used to accomplish this.

Sugar alcohols - *by ion-exchange chromatography*

A sugar alcohol (also known as a polyol, polyhydric alcohol or polyalcohol) is a hydrogenated form of carbohydrate, whose carbonyl group (aldehyde or ketone, reducing sugar) has been reduced to a primary or secondary hydroxyl group. Disaccharides and monosaccharides can both form sugar alcohols; however, sugar alcohols derived from disaccharides (e.g. maltitol and lactitol) are not entirely hydrogenated because only one aldehyde group is available for reduction.

In this trial, the separation of seven sugar alcohols on an Agilent Hi-Plex Ca column was improved by introducing acetonitrile into the mobile phase. A solution of the sugar alcohols was made up to contain 10 mg/mL of isoerythritol, adonitol, arabitol, mannitol, xylitol, dulcitol and sorbitol in water.

When pure water is used for the mobile phase, several of the sugar alcohols in the sample either partially, or completely, co-elute. Modifying operating temperature or flow rate is very unlikely to give a good separation between these compounds. Introducing acetonitrile into the mobile phase has a significant effect on the selectivity of the Hi-Plex Ca material, and results in a doubling of the retention time (Figure 21).

Column:	Hi-Plex Ca USP L19 8 µm, 250 x 4.0 mm (Part No. PL1570-5810)
Inj Vol:	10 µL
Temp:	90 °C
Detection:	Agilent PL-GPC 50 (RID)
Mobile Phase:	30:70 Acetonitrile:water
Flow Rate:	0.30 mL/min

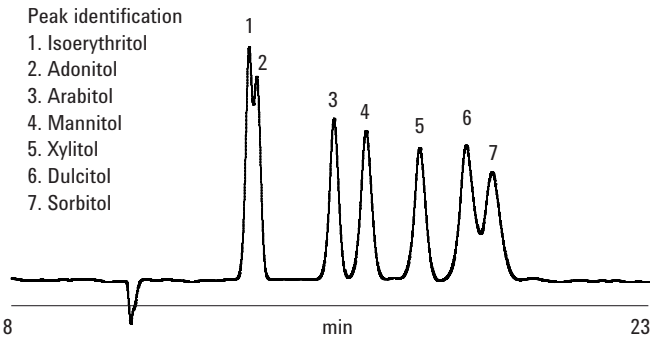

Figure 21. *Raw-data chromatogram of seven sugar alcohols on the Agilent Hi-Plex Ca USP L19 column after the introduction of acetonitrile into the mobile phase*

Using 30% acetonitrile gives an extra retention for sugar alcohols and, as a result, increases the resolution between them. All seven sugar alcohols are partially, or completely, separated.

Sorbitol - *by ion-exchange chromatography*

Sorbitol, also known as glucitol, is a sugar alcohol that the body metabolizes slowly. It is obtained by reduction of glucose, during which the aldehyde group is changed to an additional hydroxyl group. As an excipient, sorbitol acts as humectant and plasticizer in the shell of capsules, to keep the shell pliable so that it is easy to manipulate and does not dry out.

According to the USP method, sorbitol is analyzed using a liquid chromatograph equipped with a refractive index detector maintained at about 50 °C and a 7.7 mm x 10 cm column that contains packing L34 (a strong cation-exchange resin consisting of sulfonated cross-linked styrene/divinylbenzene copolymer in the lead form). Column temperature should be maintained at 50 °C with a flow rate of 0.7 mL/min. A solution of sorbitol and mannitol, used as an internal standard, was made up to contain 4.8 mg/mL of each compound. The requirement for this assay is that the relative retention times are about 0.6 for mannitol and 1.0 for sorbitol, with a resolution of not less than 2.0 between them. The analysis was accomplished using an Agilent Hi-Plex Pb USP L34 column designed for this application (Figure 22).

Column:	Hi-Plex Pb USP L34 8 µm, 100 x 7.7 mm (Part No. PL1170-2820)
Mobile Phase:	Water
Flow Rate:	0.7 mL/min
Temp:	50 °C
Detection:	RI

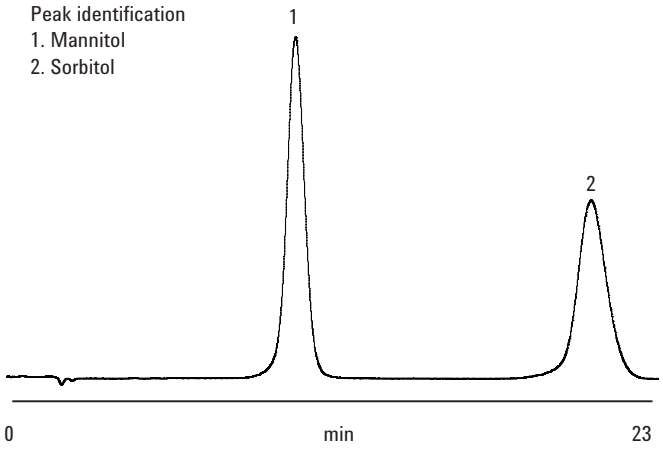

Figure 22. *Raw-data chromatogram of sorbitol and mannitol on an Agilent Hi-Plex Pb USP L34 column*

Mannitol - *by ion-exchange chromatography*

Mannitol is a polyol sugar alcohol that is used as an osmotic diuretic agent and a weak renal vasodilator. It is a sorbitol stereoisomer. Chemically, mannitol is a sugar alcohol, or a polyol, which is similar to xylitol and sorbitol.

According to the USP method, mannitol is analyzed using a liquid chromatograph equipped with a refractive index detector maintained at a constant temperature and a 4 mm x 25 cm column that contains packing L19 (a strong cation-exchange resin consisting of sulfonated cross-linked styrene/divinylbenzene copolymer in the calcium form). Column temperature should be maintained between 30 °C and 85 °C with a flow rate of 0.5 mL/min. A solution of mannitol and sorbitol, used as an internal standard, was made up to contain 4.8 mg/mL of each compound. The only requirement for this assay is that the resolution between mannitol and sorbitol is not less than 2.0. An Agilent Hi-Plex Ca USP column was used for the analysis (Figure 23).

Column:	Hi-Plex Ca USP L19 8 µm, 250 x 4.0 mm (Part No. PL1570-5810)
Mobile Phase:	Water
Flow Rate:	0.5 mL/min
Temperature:	70 °C
Detection:	RI

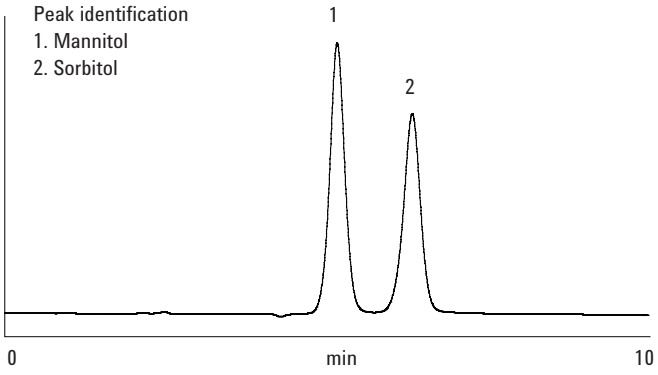

Figure 23. *Raw data chromatogram of mannitol and sorbitol on an Agilent Hi-Plex Ca USP L19 column*

Suspending and/or viscosity increasing agents

Suspension and viscosity-increasing excipients such as hydroxyethyl cellulose are used to uniformly disperse other ingredients throughout a formulation, and maintain their suspension so that actives do not precipitate or settle under gravity. This is particularly valuable for liquid formulations, during and after manufacture.

Hydroxyethyl cellulose in organic eluent

Hydroxyethyl cellulose (HEC) is widely used by the cosmetic and pharmaceutical industries, for example, as a carrier gel for microbiocides. It is a nonionic polymer with many useful properties as a thickening agent, stabilizer, emulsifier or dipersant, and it easily dissolves in hot and cold water.

HECs can be analyzed by aqueous GPC but very often they are soluble in polar organic solvents, such as dimethyl formamide (DMF). PLgel 5 µm MIXED-C columns are well suited to the analysis of these celluloses. LiBr modifier is added to minimize sample aggregation as some of these materials are ionic (Figure 24). PEO/PEG standards are used as calibrants; polystyrene is soluble in DMF, but some adsorption is apparent. Table 3 shows the dispersity and molecular weight averages of three samples of hydroxyethyl cellulose.

Table 3. *Molecular weight averages and dispersity of three hydroxyethyl celluloses*

Sample	Molecular weight average			Polydispersity (Mw/Mn)
	Mn	Mw	Mp	
A	27,000	140,000	80,000	5.2
B	30,000	159,000	102,000	5.2
C	39,000	345,000	190,000	8.9

Columns: PLgel 5 µm MIXED-C, 300 x 7.5 mm
(Part No. PL1110-6500)
Eluent: DMF + 0.1% LiBr
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System

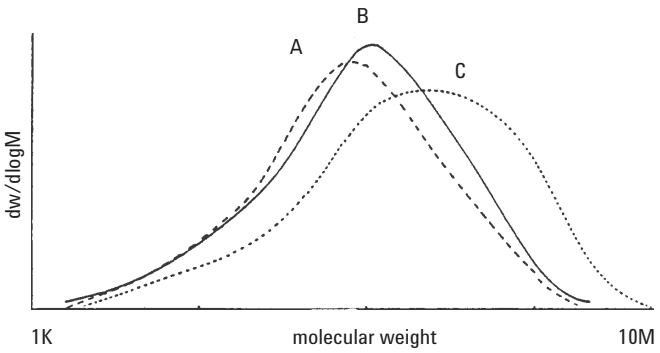

Figure 24. *Analysis of three samples of hydroxyethyl cellulose using Agilent PLgel 5 µm MIXED-C columns*

These three materials were quite different in molecular weight, indicating potential performance differences in end-use.

Hydroxyethyl cellulose in aqueous eluent

Three samples of hydroxyethyl cellulose were analyzed by size exclusion chromatography using PL aquagel-OH columns. The calculated molecular weight averages were compared with manufacturers' quoted viscosity values. Calibration was done using pullulan polysaccharide standards, also from Agilent. Figure 25 shows the raw-data chromatograms for a mixture of hydroxyethyl celluloses. A good correlation between viscosity and molecular weight averages was obtained, as can be seen in Table 4.

Table 4. *Molecular weight averages and viscosity ranges of three hydroxyethyl celloloses*

Sample	Molecular weight average			Viscosity range (cps)
	Mn	Mw	Mz	
A	60,300	179,000	139,000	75 to 112
B	413,000	849,000	1,552,000	250 to 324
C	914,000	2,016,000	3,422,000	1,500 to 2,500

Columns: PL aquagel-OH 60 8 µm, 300 x 7.5 mm
(Part No. PL1149-6860)
PL aquagel-OH 40 8 µm, 300 x 7.5 mm
(Part No. PL1149-6840)
Eluent: 0.05 M NaH_2PO_4 + 0.25 M NaCl at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

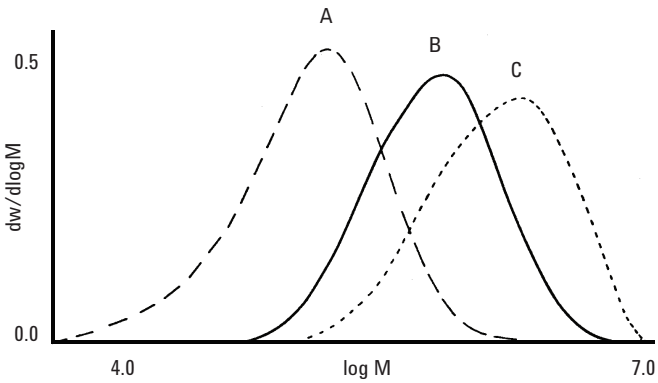

Figure 25. *Raw-data chromatograms for a mixture of hydroxyethyl celluloses on Agilent PL aquagel-OH columns*

Modified hydroxyethyl cellulose

Modifying the hydrophobicity of HEC alters the molecular weight, and such changes can be assessed by size exclusion chromatography with PL aquagel-OH 40 and PL aquagel-OH 60 8 µm columns from Agilent.

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. Column calibration was achieved using Agilent pullulan standards.

Figure 26 shows overlaid molecular weight distributions of a sample of HEC before and after modification to its hydrophobicity. Sample A is HEC. Sample B is Sample A after hydrophobic modification.

Samples: Hydroxyethyl cellulose before and after modification
Columns: PL aquagel-OH 60 8 µm, 300 x 7.5 mm
(Part No. PL1149-6860)
PL aquagel-OH 40 8 µm, 300 x 7.5 mm
(Part No. PL1149-6840)
Eluent: 0.05 M NaH_2PO_4 + 0.25 M NaCl at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
System: PL-GPC 50 Integrated GPC/SEC System (RI)

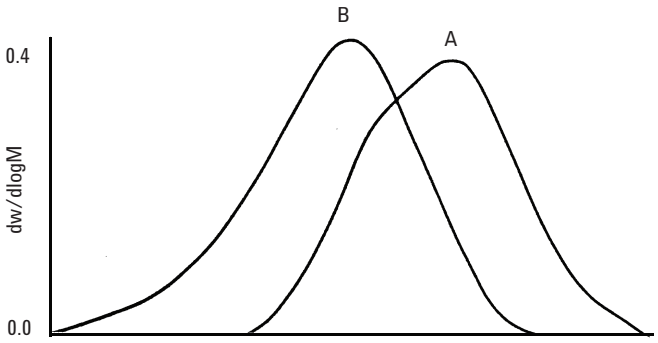

Figure 26. *Overlaid molecular weight distributions of a sample of hydroxyethyl cellulose before and after modification*

More Agilent solutions for excipients

Agilent offers an extensive toolkit for all aspects of excipient analysis. For physicochemical characterization, look to spectroscopic techniques such as NMR, FTIR, and mass spec. For chromatographic characterization we recommend HPLC, GC/MS and SEC. If you want to assess impurities then AAS, GC/MS and UV-Vis methods are appropriate. To measure physicochemical properties use dissolution testing and refractive index detection.

GPC-FTIR

Interfacing chromatographic methods with other analytical techniques can significantly increase the amount of information available for excipient characterization. Agilent offers two innovative interfaces to couple gel permeation chromatography (GPC) with Fourier transform infrared spectroscopy (FTIR), enabling rapid determination of compositional heterogeneity and its relationship to molecular weight from a single measurement.

GC/MS

For volatile impurities and residual impurities, Agilent provides the broadest selection of gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) systems, support, and supplies in the industry. So whether you need flexible, reliable hardware and software for complex research; simple, robust systems for routine production environments; or fast, rugged portable solutions for real-time measurements in the plant or in the field, we have a GC or GC/MS to meet your analytical and business challenges.

LC and LC/MS

These are ideal techniques for assessing semi-volatile impurities in excipients. For basic analysis or confirmation (confirm by known molecular weights) use the Agilent 6100B Series Single Quadrupole, or Agilent 6200 Accurate-Mass TOF LC/MS systems with Easy Access software. For more complex structural analysis of unknown excipients the Agilent 6500 Accurate-Mass Q-TOF LC/MS systems provide superior data quality and advanced analytical capabilities to profile, identify, characterize, and quantify excipients. Couple with MassHunter software tools for complete confidence.

Agilent Poroshell 120 columns provide rugged, high resolution separations within the pressure range of any mainstream LC, making the benefits of sub-two-micron performance available with existing LCs. Poroshell columns can achieve high resolution and high speed separation on current instruments, and higher resolution and speed on new high-pressure LC and LC/MS systems.

Agilent Poroshell 120 columns

ICP-MS

For heavy metals impurities, the Agilent 7700x ICP-MS is configured for routine analysis of high-matrix samples, and features Agilent's unique high-matrix interface (HMI), and ORS3 cell. With its high-temperature plasma (resulting in low oxide interferences), matrix tolerant interface and 9 orders dynamic range, the 7700x provides the ideal combination of robustness, sensitivity and analytical range required from a workhorse instrument while retaining the flexibility to handle more advanced research applications.

Dissolution testing

For Apparatus 1, 2, 5 and 6 requirements use the Agilent 708-DS Dissolution Apparatus and for Apparatus 3 the Agilent BIO-DIS Reciprocating Cylinder Apparatus. We recommend the Agilent Reciprocating Holder Apparatus 7 for Apparatus 7 use, with the Agilent 400-DS Apparatus 7 for small volume formulations.

Flash for drug discovery

The Agilent 971-FP Flash Purification System is a completely dedicated, high throughput and high recovery system for sample purification at the drug discovery phase of the value chain. Designed with chemists in mind, the system achieves maximum recovery and the highest purity in the shortest possible time. For chemists performing intermediate compound purification, the easy-to-use and 'open access' 971-FP enables a large team to work independently, improving productivity in the purification and recovery of drug candidate compounds.

Using the robust 971-FP instrument with IntelliFlash software, DASi (Dissolve, Absorb, Sample injection) module, and SuperFlash purification columns, equips you with an advanced set of method development tools designed to reduce system set-up time and quickly identify the optimum purification method. For development scale purifications, Agilent has the range of larger Flash F75/F150 cartridges.

Agilent SuperFlash columns

The Agilent 971-FP Flash Purification System

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 PL aquagel-OH 40 8 µm, 300 x 7.5 mm	PL1149-6840
Agilent PL aquagel-OH 50 8 µm, 300 x 7.5 mm	PL1149-6850
Agilent PL aquagel-OH 60 8 µm, 300 x 7.5 mm	PL1149-6860
Agilent PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm	PL1149-6800
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 Hi-Plex Ca USP L19 8 µm, 250 x 4.0 mm	PL1570-5810
Agilent Hi-Plex Pb USP L34 8 µm, 100 x 7.7 mm	PL1170-2820
Agilent PLRP-S 100Å 5 µm, 150 x 4.6 mm	PL111-3500

Instruments	
Description	Part No.
Agilent 1260 Infinity GPC/SEC Analysis System	Contact local sales office
Agilent PL-GPC 50 Integrated GPC/SEC System	G7810A
Agilent 380-LC ELSD	G4260A
Agilent 1260 Infinity GPC/SEC Multi Detector Suite	G7800A

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
Elastomers	5990-6866EN
Polyolefin analysis	5990-6971EN
Low molecular weight resins	5990-6845EN
Food additives	5990-8634EN
Organic GPC/SEC columns product guide	5990-7994EN
Aqueous and polar GPC/SEC columns product guide	5990-7995EN
GPC/SEC standards product guide	5990-7996EN

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