GPC/SEC Start-up Kit

The Measure of Confidence

Agilent Technologies

GPC/SEC Start-up Kit

Congratulations for choosing GPC/SEC solutions from Agilent. This kit contains everything you need to get you up and running quickly and easily.

The information in this booklet will provide you with the knowledge to get the most out of your GPC separations.

Table of contents

Organic GPC/SEC Start-up kit

Part number PL2010-0700

Description	Part number	Quantity
PLgel 5 µm MIXED-C, 7.5 x 300 mm	PL1110-6500	1
2 mL vials of EasiVial PS-M	PL2010-0301 (30/pk)	9
Broad polystyrene standard		3
Literature		
PLgel data sheet		1
EasiVial polystyrene 2 mL guide		1
Certification		
Column performance certificate		1
EasiVial 2 mL certificate of analysis		1
Broad polystyrene certificate of analysis		1

Aqueous GPC/SEC Start-up kit

Part number PL2080-0700

Description	Part number	Quantity
PL aquagel-OH MIXED-H, 7.5 x 300 mm	PL1149-6800	1
2 mL vials of EasiVial PEG/PEO	PL2080-0201 (30/pk)	9
Broad dextran standard		3
Literataure		
PL aquagel-OH data sheet		1
EasiVial PEG/PEO 2 mL guide		1
Certification		
Column performance certificate		1
EasiVial 2 mL certificate of analysis		1
Broad dextran certificate of analysis		1

Quick start guide

1. Ensure you have read and understood the enclosed data sheets.

2. Referring to the EasiVial guide, prepare the vials ahead of time using the mobile phase chosen to dissolve the standards. Vials should be left a minimum of 1 hour, but ideally overnight, to dissolve. Do not vortex or sonicate as this may damage the standards.

3. Attach the column to your system and condition your column for a minimum of 3 column volumes (approximately 45 minutes at 1.0 mL/min) using:

i. Tetrahydrofuran (THF) for organic start-up kits
ii. Water for aqueous start-up kits

4. Place one red, one green and one yellow EasiVial into your autosampler. Each vial contains a cocktail of 4 different MW standards.

5. Set up your software parameters: Software packages vary considerably depending on the supplier. Refer to your supplier's technical support if assistance is required to program your system.

6. Run your samples at 1.0 mL/min until all 12 standards have eluted. Each vial will take ~12 minutes to complete.

7. Using your software package, extrapolate the data to produce a 12-point calibration curve. The standards should give linear calibration data approximating that shown in the EasiVial guide.

8. To verify the data/test system, prepare the broad standard and run under same conditions as above.

9. Analyze the data and compare with the broad standard certificate of analysis. Whilst some system-to-system variation is to be expected the results should be within ~20% of the certified values.

NOTE:

PEG/PEO standards should be stored refrigerated until required and then bought to room temperature.

EasiVial are stable in the format shipped but will gradually degrade when dissolved. It is recommended that organic EasiVial be prepared on a weekly basis and aqueous EasiVial be prepared every 48 hours.

For technical queries, visit **www.agilent.com/chem/techsupport**

GPC Overview

Gel permeation chromatography/size exclusion chromatography (GPC/SEC) uses the liquid present in the pores of beads as the stationary phase, and a flowing liquid as the mobile phase.

The mobile phase can flow between the beads and also in and out of the pores in the beads.

The separation mechanism is based on the size of the polymer molecules in solution.

There are several names given to different types of SEC, but all are based on the same principle, that of size exclusion, hence size exclusion chromatography.

Historically, the porous medium was made of a gel and therefore gel permeation chromatography was coined, a term still prevalent in the industry today.

Low pressure analysis of biological compounds is often referred to as gel filtration chromatography (GFC).

For our purposes, SEC and GPC refer to the same instrumentation and column technology.

Polymers

Polymers have many useful physical properties including hardness, thermal and electrical insulation, and resistance to chemicals. These parameters are influenced by the polymer chemistry, molecular structure and shape, molecular weight, and the presence of branching. Furthermore, these parameters can be characterized by examination of the finished product or be predicted from an understanding of the polymer molecules.

Polymers are composed of many repeating units joined together. The term is derived from the Greek words polloi (many) and meros (parts). There are a large number of natural polymers from plants and animals, such as rubber, polysaccharides, starch, cellulose and glycogen. Proteins, nucleic acids and some inorganic large molecules can also be thought of as polymers.

The chemical basis for the formation of polymers is the ability of the single, or 'monomer', units to form long chains. Many molecules can do this, leading to the development of many different types of man-made polymers. The chemical reaction when polymers join together is called polymerization.

Take, for example, polyethylene – made up of repeating units of ethylene $(C_2H_4)n$, where n can be a very large number.

The interesting thing about polymers is that the length of the molecular chains can be shorter or longer and the compound will still be recognizable as the same polymer. In practice, a sample of polymer will contain a distribution of molecules of different lengths.

Polymer chains

Plastics, such as those used to make polyethylene bags, polystyrene foam cups, and polypropylene drain pipes, are made by linking monomers together to form chains. Many of the useful properties of plastics, such as mechanical strength and elasticity, come from the intertwining of these long molecular chains. Generally, the longer the chains, the more intertwined they are, and therefore the harder and tougher the material will be. So, depending on the chain lengths in a sample of polyethylene, the material could be liquid, a wax, or a rigid solid, with its physical state obviously having a major impact on how it is used. In this case, the chemistry of these materials is the same – they're all polyethylene – it's just the physical state of the materials that differs. Furthermore, all synthetic polymers contain a distribution of polymer chain lengths; in fact, it's impossible to make polymers in which all the chains are the same length. GPC/SEC is a technique that allows you to separate out the different lengths of polymer chain in a sample and measure their relative abundance.

GPC/SEC is a technique for measuring the chain lengths and other characteristics of polymers by separating them on the basis of their size. It's as simple as that!

How does GPC/SEC work?

A GPC/SEC instrument consists of a pump to deliver the solvent through the instrument, an injection port to introduce the sample onto the column, a column to hold the stationary phase, one or more detectors to detect the components as they leave the column, and software to control the different parts of the instrument and calculate and display the results.

Firstly, the polymer sample is dissolved in a solvent. This is an important step, because although polymer molecules can be described as long chains of monomers linked together, they don't exist like that in solution. Once they have been dissolved, the molecules coil up on themselves to form a random conformation, which resembles a ball of string. So although they are chains, when we analyze them by GPC/SEC they behave like tiny spheres, with the size of the sphere dependent on the molecular weight; higher molecular weight polymers coil up to form larger spheres. These coiled up polymer molecules are then introduced into the mobile phase and flow into the GPC/SEC column.

The dissolved polymer molecules move past the beads as the mobile phase carries them down the column. As the polymer coils move past each bead, several things can happen.

If the polymer coils are much larger than the biggest pores in the beads, they cannot enter the pores and so are carried straight past by the mobile phase. If the polymer coils are a little smaller than the biggest pores they can enter the larger, but not the smaller pores as they pass by, occupying some, but not all of the available stationary phase.

If the polymer coils are smaller than the smallest pores in the beads, then they can enter any of the pores and so can potentially occupy all of the stationary phase. As the molecules enter the column, this partitioning occurs repeatedly, with diffusion acting to bring the molecules into and back out of any pores they pass as they travel down the column. As a result, small polymer coils that can enter many pores in the beads take a long time to pass through the column and therefore exit the column slowly.

Large polymer coils that cannot enter the pores take less time to leave the column, and polymer coils of intermediate size exit the column somewhere between these examples. Thus, the way in which the samples elute from the column depends very much on the size of the pores in the beads.

The separating mechanism is shown in Figure 1. This diagram shows how different sized sample molecules can be excluded completely, partially, or not at all from entering the pores in the particles, depending on the size of the pores and of the sample molecules.

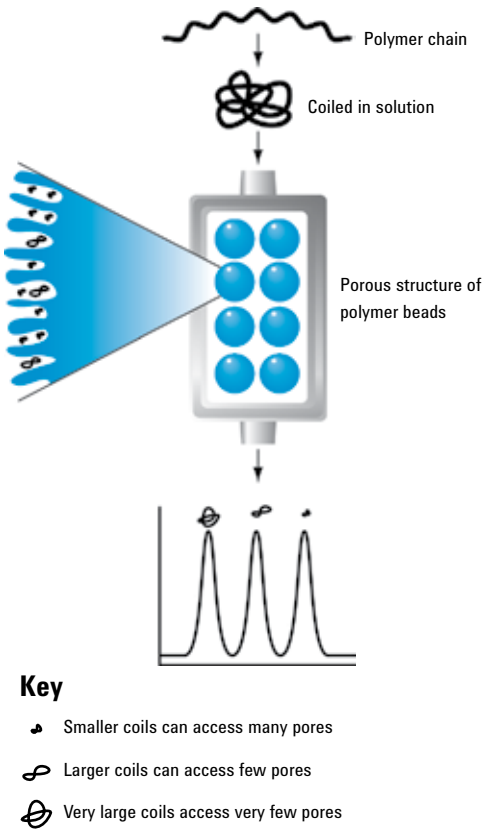

Figure 1. *How GPC/SEC separates molecules of different sizes*

- 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 on size; large coils first, smaller coils last
- The size separation data is converted to molecular weight information by using a calibration curve generated from polymer standards

As the components exit the column they are detected and the elution behavior of the sample is displayed in a graph, or chromatogram. The chromatogram shows how much material exited the column at any one time, with the higher molecular weight, larger polymer coils eluting first, followed by successively lower molecular weight (and therefore smaller) chains emerging later.

The primary separation is according to elution volume. This is converted to time for ease of measurement, on the assumption that the flow rate is constant. The time it takes for a group of molecules of the same size (a fraction) to emerge from the column is called the retention time. The sample chromatogram is then compared to a calibration curve that shows the elution behavior of a series of polymer standards, for which the molecular weight is known. This allows the molecular weight distribution of the sample to be calculated, providing important information that can be used to predict how the polymer will perform during and after processing.

One important thing to bear in mind about GPC/SEC is that the separation is based on size- and not chemistry. The information regarding the size of polymer molecules in solution is converted into molecular weights through the use of a calibration.

Who uses GPC/SEC, what for, and why?

GPC/SEC has two main uses – to characterize polymers and to separate mixtures into discrete fractions, such as polymer, oligomer, monomer and any non-polymeric additives.

GPC/SEC is the only technique available to characterize the molecular weight distribution of polymers. The polymer mixture can be separated into individual components, such as polymer and plasticizer. Naturally occurring polymers such as lignins, proteins and polysaccharides are routinely investigated using GPC/SEC in polar organic or aqueous solvents. GPC/SEC is also excellent for separations of oligomers and small molecules.

Biologists and biochemists typically use low pump pressures and columns packed with soft gels (such as polyacrylamide, dextran or agarose) to avoid damage to fragile biological compounds during chromatography. The advantage of the technique for these scientists is that the biological activity of the compounds remains unaffected and fractions can be collected for use in other experiments.

Polymer chemists and engineers in industry are more likely to use high pump pressures on columns filled with cross-linked polystyrene or silica, as this gives higher resolution and better results.

GPC/SEC is frequently used as part of a 2D-LC system in order to further separate molecules by characteristics such as their acidity, basicity, charge or affinity.

Calibrations

To determine the molecular weight distribution of a polymer sample, a calibration with standard polymers of known molecular weight must be performed. Values from the unknown sample are then compared with the calibration graph to generate molecular weights and molecular weight averages. Standards are now available in a wide range of polymer types and molecular weights, and as kits or individual molecular weights for maximum choice. Not surprisingly, standards need to be of very high quality and with extremely narrow molecular weight distributions so that the position of the top of the peak, the peak molecular weight (Mp – see Figure 3) can be assigned with confidence. It is the Mp value that is used to generate the calibration. For example, Agilent's current polystyrene at MW 1,000,000 g/mol has a very narrow distribution, or polydispersity index of 1.05 (the term polydispersity is explained in the next section).

Figure 2 shows a calibration curve from an EasiVial pre-weighed polymer standards kit from Agilent.

The molecular weight is determined from the calibration curve by simply noting the retention time (RT) of the sample, and reading the corresponding molecular weight from the vertical axis. To generate a molecular weight distribution, the peak is divided into a number of equally spaced 'slices'. The molecular weight is then determined at each data point with reference to the column calibration. Summations are used to determine the molecular weight averages– more on this below. It is also possible to use molecular weight sensitive detectors such as light scattering or viscometry to obtain more information about the polymer in solution.

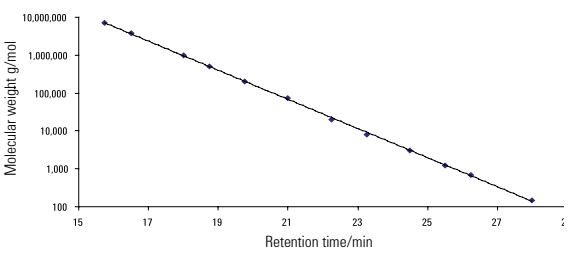

Figure 2. *A calibration graph used to determine the molecular weight of a polymer from its retention time*

For more information on calibrating your GPC columns, download the primer "Calibrating GPC Columns - A Guide to Best Practice" (5991-2720EN) at **www.agilent.com/chem/GPCresources**

Calculations in GPC/SEC

In polymers, molecular weight occurs not as a discrete value, but as a distribution. This means that to accurately assess the molecular weight distribution of a polymer, the number of particles are determined of each molecular weight. The calculated molecular weight averages are shown in Figure 3.

The number average molecular weight, is abbreviated as Mn.

Defined as the the total molecular weight of all molecules divided by the number of molecules. The value of Mn influences the thermodynamic properties of the molecule.

A second important parameter is the weight average molecular weight (Mw). Mw is defined as the value at which there are equal masses of molecules on each side, at higher and lower molecular weight. Mw is large molecule sensitive and influences the bulk properties and toughness of the polymer. Unsurprisingly, the Mw value is always greater than the Mn value unless the polymer is completely monodisperse.

Mw affects many of the physical properties of polymers, and is the most often quoted molecular weight average. As well as Mn and Mw, there are other molecular weight averages that take increasing account of the higher molecular weight components of the sample, such as z-average molecular weight (Mz) and Mz+1. Mz is sensitive to even larger molecules and influences viscoelasticity and melt flow behavior.

The ratio of Mw to Mn is used to calculate the polydispersity index (PDI) of a polymer, which provides an indication of the range of molecular mass of the material. The broader the molecular weight distribution, the larger the PDI.

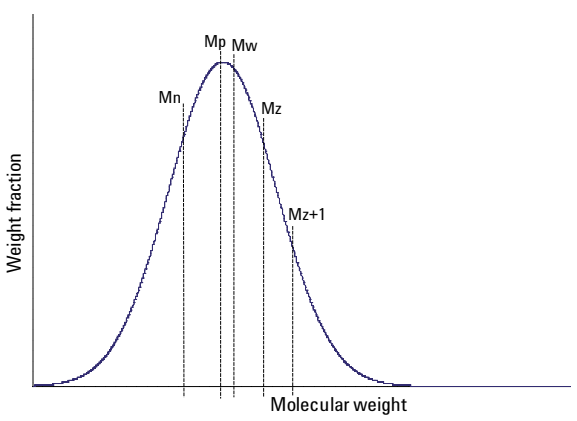

Figure 3. *The average molecular weights of a mono-modal polymer – in this case the distribution is nearly symmetrical*

However, obtaining all of these numbers is not particularly straightforward. Standard GPC/SEC detectors cannot count the number of molecules that elute from the column, so these weight averages cannot be measured directly. Nonetheless, the concentration of molecules can be measured on a weight/volume basis, and the molecular weight averages calculated by using a concentration sensitive detector, such as differential refractive index.

Substituting concentration units for numbers in the equations allows us to recalculate Mn, Mw and Mz, based on the concentration units instead. All of these averages help build a picture of the nature of a polymer and provide information on its likely behavior, as illustrated in Figure 4. Molecular weights on the horizontal scale are often expressed in log values. This is a common mathematical method of compressing the length of the axis on a graph when very large numbers are involved, since the molecular weight of polymers can exceed 10,000,000 g/mol.

The molecular weight values of a polymer are important, since they influence properties such as brittleness, toughness, and elasticity. Slight differences in these values can cause major differences in the way a polymer behaves and determine its suitability for a particular industrial use. Figure 4 illustrates how molecular weight influences the properties of a polymer.

This example illustrates that knowledge of the polymer molecular weight distribution allows chemists to predict how it will behave. This information is obviously valuable before moving from laboratory R&D scale to full commercial production because it shows if the product will be able to meet the industrial specification required.

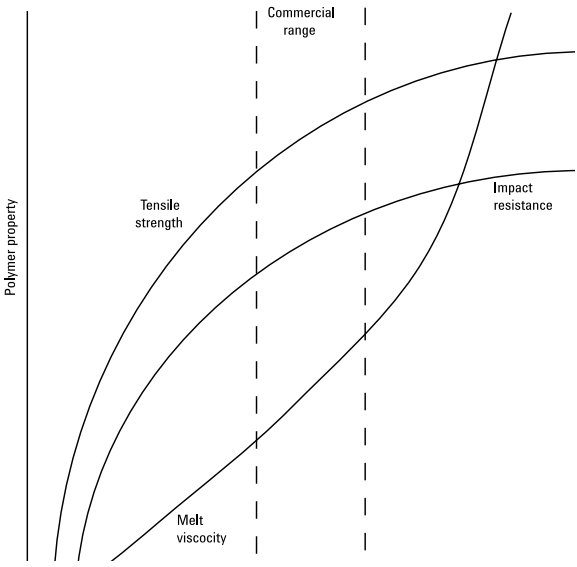

Figure 4. *The effects of molecular weight on the properties of polymers*

Types of polymer distribution

Polymer distributions can be wide with lots of high molecular weight and low molecular weight components, or narrow with most of the components grouped around the same molecular weight. In chromatographic terms this is measured by the polydispersity of the polymer. Polydispersity values are important guides to polymer behavior.

- Monodisperse polymers consist of molecules all with the same molecular weight where the values of Mn, Mw and Mz are identical. In practical terms, the only monodisperse polymers are those found in nature, such as proteins and nucleic acids.
- Synthetic narrow distribution polymers are synthesised to make all the chains as close in molecular weight as possible. The Mw/Mn polydispersity ratio of less than 1.2, is a subjective but convenient definition of a polymer with a narrow distribution. Examples include polymers used as standards for calibrations – here the top of the peak needs to be defined as accurately as possible, so having a very narrow molecular weight distribution is beneficial. In this case, the molecular weight averages are very close to each other. The distribution is nearly normal in shape, i.e. the areas under the curve on either side of the center line are equal.
- The most common synthetic polymers typically have a polydispersity ratio between 1.2 and 3, because many of the synthesis procedures used tend statistically towards these values.
- Broad distribution polymers may also be synthetic, or natural such as polysaccharide and starch carbohydrates. These distributions are rarely normal in shape; for example, they may have a long tail towards the lower molecular weight. The presence of the low molecular weight compounds shifts Mn more than Mw and Mz.
- Sometimes synthetic polymers have multimodal distributions. One example is shown in Figure 5. Importantly, the values of the molecular weight averages do not reveal in themselves that the sample is multimodal – careful observation of the molecular weight distribution is required.

Figure 5 shows molecular weight distributions for polymers of differing polydispersities. A narrow distribution consists of a single, sharp peak, with a polydispersity of <1.2. A medium distribution has a wider size range of polymer chains with a polydispersity of <2.0, over which the distribution is classed as broad. The profiles can be Gaussian, with symmetry around the highest point, but often they are weighted towards either high or low molecular weight. If two or more polymers of differing molecular weight are present, then the two peaks can overlap, resulting in a bimodal distribution.

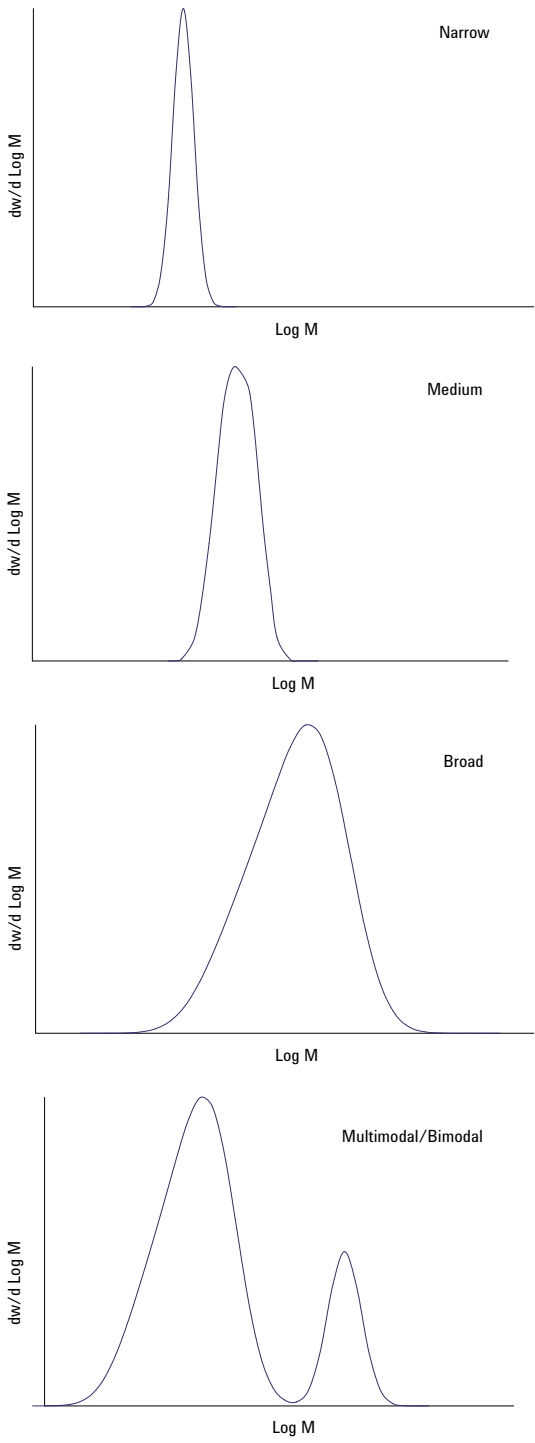

Figure 5. *Different polymer molecular weight distributions*

GPC/SEC in practice

A chromatograph has to accomplish a number of tasks, including mixing a sample with the solvent, pumping it through the column, detecting the sample fractions and capturing and displaying the results (Figure 6). This section explains more about the system components, how they function and what makes a good system.

Solvents and solvent containers

In GPC/SEC, the selection of a solvent depends on several factors. The solvent must be able to dissolve the sample, which may not be obvious at first sight; sometimes a polymer insoluble at room temperature will dissolve at higher temperature. The solvent must not induce any other interactions between the sample and the stationary phase, so that the separation is solely on the basis of sample size.

The solvent container should be made of clear glass, or amber glass for solvents affected by sunlight, with a stopper to exclude dust and limit evaporation. It is useful to purge the solvent with helium or ultrasonicate it before use, or use an inline degasser. This is because dissolved gas tends to 'out gas' in the solvent during use, forming bubbles that prevent the pump operating at the required flow rate. There is usually a filter in the solvent line to stop particulates entering the pump.

To quickly and easily find the right vials and closures for your application, use our interactive online tool **www.agilent.com/chem/SelectVials**

Ovens

GPC/SEC is usually carried out at room temperature, using organic solvents such as tetrahydrofuran (THF), chloroform or toluene. Some instruments have heated and thermostatically controlled ovens in which the columns are placed. Higher temperatures (up to 220 °C) are necessary for certain samples which have limited solubility (e.g polyolefins) and for some solvents that have much higher viscosities, such as trichlorobenzene or chloronaphthalene. Operating the instrument at high temperatures reduces viscosity and hence column back pressure, with a corresponding increase in efficiency. For samples that require elevated temperatures to remain in solution it is also necessary to house detector flow cells in an oven enclosure.

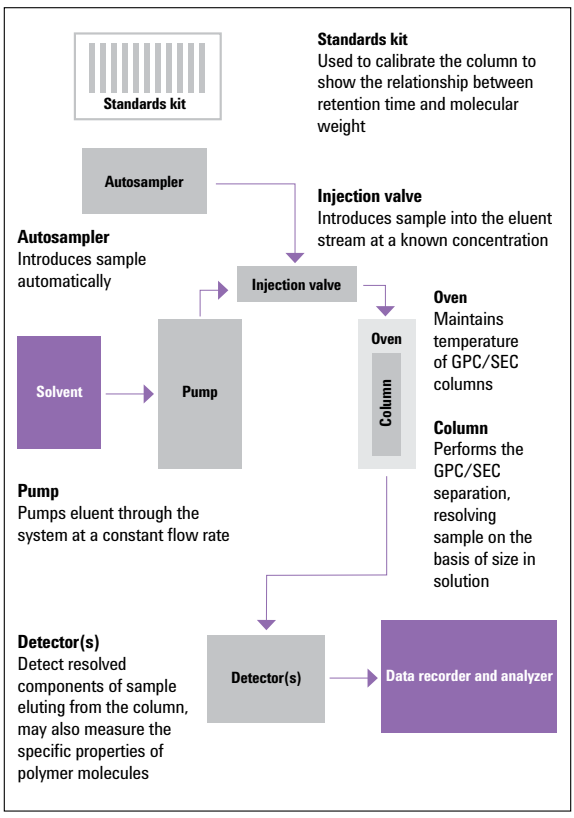

Figure 6. *The main components of a GPC/SEC system*

Samples

Sample preparation is very important in GPC/SEC, especially for large molecules. To prepare a sample for analysis it is first dissolved in an appropriate solvent, such as tetrahydrofuran (THF) for organic GPC or water based buffers for aqueous SEC. Since the separation obtained depends on the size of the sample molecules, it is important that they are allowed to swell and then fully dissolve in the solvent (which may take several hours) prior to analysis.

Where possible, the solvent used to prepare the samples should be the same as the eluent running through the system. Sample concentration employed during analysis is dependent on the molecular weight and the viscosity of the sample under investigation. The table below gives some typical sample concentrations for analytical GPC/SEC.

Sample molecular weight (g/mol)	Sample concentration % (weight to volume)
< 5000	< 1.0
5,000 to 25,000	< 0.5
25,000 to 400,000	< 0.25
400,000 to 2,000,000	< 0.20
10,000,000	< 0.05

Injection and injectors

Injectors introduce the polymer sample into the flowing solvent stream without interrupting the flow. Injection volumes are generally in the range of 20 to 200 µL. It is important that the injector does not disturb the flow of the mobile phase.

Autosamplers, sometimes with heaters and filters to dissolve and clean the sample, reduce the work involved if many samples are needed or when the sample volume is large.

Columns and column sets

The columns vary in length from 50 to 600 mm, and internal diameters of 4.6 to 25 mm. For example, the smaller columns (7.5 x 50 mm id) are used as guard columns, mid sizes (4.6 or 7.5 x 300 mm) for analytical work, and the largest (25 x 600 mm) for semi-preparative chromatography.

The type of particle in the column is controlled to match different applications. For example, organic GPC columns from Agilent are filled with cross-linked polystyrene/divinylbenzene beads with particle sizes from 3 to 20 µm. The pore sizes range from 50 to 1,000,000Å. The pore 'size' is defined as the length of the polystyrene molecule in its natural, state, that when dissolved in tetrahydrofuran and coiled in a sphere would only just fit in the pore – it is not the actual physical diameter of the pore. As opposed to HPLC, the pore size is the actual dimension of the pore.

There are a range of columns available which are packed with a variety of particles with a range of pore sizes to characterize polymers which have different molecular weight ranges. The nature of the sample and solvent determines which column configuration will provide the best analytical result, which explains why there are many different combinations of particle size and pore size – one size won't fit all (a more detailed explanation is given in the FAQs chapter).

Although there are many different column packings, there are fewer column formats, just variations of analytical, preparative and guard columns. **Columns are usually employed in combinations of two or three columns to improve the resolution of the system.** Guard columns are often used before the main column. As its name implies, the guard column protects the main column by stopping insoluble particles or contaminants that could block the main column set.

Pumps

The pump delivers the solvent at a constant, accurate and reproducible flow rate. The pump has to be able to run the same flow rate regardless of viscosity, so that results can be compared from one analysis to another. The pressure delivered by the pump also needs to be smooth so that there are no pulses in the flow. Using a different solvent means flushing the system, and so the internal volume of the pump should be small so that solvent is not wasted.

Pumps are manufactured with the wettable parts made from inert materials such as stainless steel, titanium and ceramics, which do not react with the solvents used in GPC/SEC.

Detectors

Chromatography uses the chemical and physical properties of sample molecules and mobile phases to detect their presence as they elute from a column, and so different detectors have been developed that make use of the different characteristics of compounds. The instruments also need to have a wide sensitivity range so that they react accurately both to trace quantities and large amounts of material, if necessary. Detectors may respond to a change in the mobile phase due to the presence of the sample, or to a property of the sample alone. The former have to be very sensitive since the changes they measure in the mobile phase are very small. The latter have much greater sensitivity but often only work with specific samples. The ability to scatter light, molecular viscosity and the adsorption of ultraviolet (UV) or infrared (IR) are all used as measurement parameters. Detectors can be divided into those that measure concentration alone, such as differential refractive index (DRI), UV and evaporative light scattering (ELS) detectors, and those whose response is proportional to concentration and other properties of the polymer molecules, such as static light scattering detectors or viscometers.

The most common GPC detector is based on the principle of refractive index. These detectors work by assessing the difference in refractive index between the mobile phase and the pure solvent, so they are known as differential refractive index detectors. DRIs are sometimes referred to as 'universal' detectors, as they tend to give a usable response for all types of polymer. Since the refractive index of polymers is usually constant above molecular weights of about 1,000 g/mol, the detector response is directly proportional to the sample concentration.

Viscosity detectors also compare the mobile phase with the pure solvent, only this time using viscosity, or resistance to flow, as the measurement parameter. These instruments provide accurate molecular weight determination, and information on how dense the polymer molecules are, which is not available from normal concentration detectors.

Static light scattering detectors use the fact that a beam of light will be scattered when it strikes a polymer molecule. There are various types, including low angle laser light scattering (LALLS), multi-angle laser light scattering (MALLS) and right angle laser light scattering (RALLS). The advantage of these detectors is that they give a response directly proportional to the molecular weight of the polymer molecules, and can provide size information too.

Conventional GPC/SEC

A conventional instrument for GPC/SEC consists of a precision solvent delivery system, an automatic injection valve, a column, a concentration detector such as a high performance DRI, and software to control all the hardware, capture the data, conduct the analysis and display the results.

The Agilent PL-GPC 50 Integrated GPC/SEC System, is such an instrument.

Multi-detector GPC/SEC

To gain the maximum benefit from a GPC/SEC analysis, it is best to use different detectors to extract all the potential information from the sample. This can be achieved by using multiple detection with concentration, viscometry and static light scattering detectors in the same GPC/SEC instrument.

If all detectors are used, then the technique is called triple detection. Multi-detector options allow accurate molecular weights to be readily determined via analysis that remains independent of column calibration, while the analysis of structural properties, such as the number of branches on the polymer backbone, is possible through the measurement of molecular sizes.

Automatic data processing

Data management software such as Agilent GPC/SEC Software calculates and stores the values of Mn, Mw, Mz and polydispersity (Mw/Mn). This versatile software also provides complete control of GPC/SEC systems and multi-detectors so that large numbers of samples can be run unattended. GPC analysis software is also available for Agilent OpenLAB CDS ChemStation and EZChrom chromatography data systems.

FAQs

Q. Why can't I buy one column for everything?

A. There are many columns available for GPC/SEC, characterized by several key differences – the chemistry of the packing material, the resolving range of the column and the particle size of the packing. So why are there so many different types of column?

- **Chemistry of the packing material** – the chemistry of the packing material is a prime consideration. Thus Agilent PLgel and Agilent PlusPore columns are packed with beads of polystyrene/divinylbenzene copolymer, Agilent PL aquagel-OH has beads of a proprietary hydrophilic material with a polyhydroxy surface, and Agilent PolarGel is packed with macroporous copolymer material with a surface of balanced polarity. The chemistry of the column packing determines its solvent compatibility. PLgel is designed for use with organic solvents such as THF, PL aquagel-OH for water based solvents, and PolarGel for polar organic solvents and solvent mixtures. Due to the wide range of solvents there is no single column chemistry that offers the best separations in every solvent; columns must be selected to suit individual applications.
- **Resolving range** – GPC/SEC columns are available with a wide variety of resolving ranges, from a few thousand g/mol (PLgel 3 µm 100Å) to 100,000,000 g/mol (Agilent PLgel Olexis). Matching pore size and resolving range of the column with the application are key to obtaining quality results. For example, choosing a column with too large a resolving range results in too low resolution, as only a small percentage of the total pores present are the correct size to separate out the sample molecules. Conversely, choosing a column where some of the sample falls outside the molecular weight resolving range of the column results in unresolved components. Choosing a column that focuses pores over the molecular weight range of the sample ensures that the majority of the pores are involved in the separation and the resolution is maximized.
- **Particle size** – the range of particle sizes in GPC/SEC columns varies between 3 and 20 µm. Generally, smaller particles mean improved resolution for the same column length and faster separation with the same resolution. That is why we recommend, for example, PLgel 3 µm 100Å columns for GPC/SEC separations of low molecular weight compounds such as mono-, di- and triglycerides, and linear hydrocarbons. However, when separating larger, diffuse molecules, such as polyolefins, it is important to choose a large particle size to avoid shear degradation. PLgel Olexis is the column of choice for such applications.

Q. I've run my column dry or used an inappropriate solvent – can I save it?

A. Sometimes you can, depending on whether the gel bed has remained stable. Disconnect the column and flush fresh solvent back into the GPC/SEC system, at a flow rate of 0.2 mL/min. The solvent used should be the same as the running solvent, without any salts or additives. Once the system is back into solvent, the column can be reintroduced and flushed into the new solvent at reduced flow, typically at one-fifth the running flow rate (0.2 mL/min for a column running at 1.0 mL/min). Flush for at least three column volumes, then increase the flow to the run flow rate and flush for a further three column volumes.

Flushed into an inappropriate solvent – follow the dry run procedure, using a solvent compatible with the column packing. However, it is important that the solvent used is fully miscible with the solvent in the column. In case of doubt, acetone is a good intermediate solvent for organic columns, while for aqueous columns, water must be used.

Testing – once these procedures have been performed, it is important to test the column by repeating a known separation to ensure the column has not been irreversibly damaged.

Recommendations for setting up a GPC/SEC system

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

Choosing an eluent for GPC/SEC

Question 1. What is the sample soluble in?

Many polymers are only soluble in a small number of solvents. This is the key question when developing methods for analyzing polymers. The solvents mentioned here are all common eluents employed in GPC/SEC.

Answer	Recommendation	Comments
Water or water buffer with up to 50% methanol	Agilent PL aquagel-OH	Best choice for water-based applications but cannot accommodate organics apart from methanol up to 50%
Typical organic solvent such as THF, chloroform, toluene	Agilent PLgel or Agilent PlusPore	PLgel are the workhorse columns, PlusPore columns are an alternative
Organic/water mixtures or polar organics such as, DMF, NMP	Agilent PolarGel	PolarGel is a smaller column range than PLgel or PL aquagel-OH columns but is suited to mixtures of organics and water

Choosing a column for GPC/SEC

*Standards shown in **Bold** are the best initial choice*

Question 2. 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.

Answer	Recommendation	Comments
High (up to several millions)	Aqueous solvents **PL aquagel-OH MIXED-H 8 µm** or combination of PL aquagel-OH 40 and 60 15 µm	The 15 µm column combination is best only where sample viscosity is very high, otherwise 8 µm columns give greater resolution
	Organic solvents **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
	Mixed solvents **PolarGel**	No PolarGel column available for this molecular weight range. Contact your local GPC/SEC expert for advice
Intermediate (up to hundreds of thousands)	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	A wide-ranging column that covers most water-soluble polymers
	Organic solvents **PLgel 5 µm MIXED-C** or PLgel 5 µm MIXED-D, PolyPore or ResiPore	The PLgel columns are the most widely applicable for the majority of applications; PolyPore and ResiPore columns are alternatives
	Mixed solvents **PolarGel-M**	Covers most applications
Low (up to tens of thousands)	Aqueous solvents Combination of **PL aquagel-OH 40 and PL aquagel-OH 30 8 µm**	These two columns in a combined set cover the low end of the molecular weight range
	Organic solvents **PLgel 3 µm MIXED-E** or MesoPore	The PLgel column provides high resolution and is designed for low molecular weight applications; the MesoPore column is an alternative
	Mixed solvents **PolarGel-L**	For low molecular weight applications
Very low (a few thousand)	Aqueous solvents **PL aquagel-OH 20 5 µm**	This high-performance column gives high resolution at low molecular weight
	Organic solvents **OligoPore** or PLgel 3 µm 100Å	The OligoPore column is less prone to dispersion than the PLgel column, but both work well
	Mixed solvents **PLgel**	No PolarGel column covers this range so use PLgel columns as alternatives
Unknown	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	Covers the molecular weight ranges of most polymer samples
	Organic solvents **PLgel 5 µm MIXED-C** or PolyPore	This PLgel column is the most widely applicable for the majority of applications
	Mixed solvents **PolarGel-M**	Covers the majority of applications

Setting up the GPC/SEC system

Question 3. 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.

Answer	Recommendation	Comments
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 efficiencies
	Particle size 13 µm use 3 columns	
	Particle size 10 µm use 3 columns	
	Particle size 8 µm use 2 columns	
	Particle size 5 µm use 2 columns	
	Particle size 3 µm use 2 columns	

Question 4. What size injection volume?

The injection volume required is dependent on the particle size of the column – smaller particles need lower injection volumes to minimize dead volume. Larger injection volumes allow the introduction of high molecular weight samples at lower concentrations, reducing viscosity and ensuring a quality chromatogram is obtained.

Answer	Recommendation	Comments
Depends on the particle size of the columns	Particle size 20 µm use 200 µL injection	Smaller particle sizes require smaller loops to minimize band broadening
	Particle size 13 µm use 200 µL injection	
	Particle size 10 µm use 200 µL injection	
	Particle size 5 µm use 100 to 200 µL injection	
	Particle size 3 µm use 20 µL injection	

What standards should I use?

*Standards shown in **Bold** are the best initial choice*

Question 5. What is the eluent?

Standards are polymers, so the choice of standard mainly reflects solubility in the chosen eluents.

Answer	Recommendation	Comments
Water or water buffer with up to 50% methanol	**Polyethylene glycol (PEG)/oxide (PEO)** or polysaccharides (SAC)	These standards perform in all water-based systems in convenient Agilent EasiVial format
Typical organic solvent such as THF, chloroform, toluene	**Polystyrene (PS)** or polymethylmethacrylate (PMMA)	Polystyrene is the most commonly used standard in convenient EasiVial format
Organic/water mixtures or polar organics such as DMF, NMP	**Polyethylene glycol/oxide** or polymethylmethacrylate	Polar standards perform well

Question 6. What format of standards are recommended?

Different formats of standards are available depending on customer preference.

Answer	Recommendation	Comments
For the quickest and simplest approach where accurate concentrations are not required	Easiest option – **EasiVial** or Agilent EasiCal	Simple to use, EasiVial preferred before EasiCal because of the wider choice of polymer types
If accurate concentrations are required	Accurate concentrations required – **EasiVial** or individual standards	Both formats allow accurate sample concentrations, EasiVials are simpler to use

Typical polymer molecular weights

If you are unsure of the molecular weight of your sample, the table below shows some approximate molecular weight ranges for common polymers, which will help you select the right column for your application.

Polymer Type	Typical molecular weight of polymer	Typical poldispersity[1] of polymer
Polymers from free radical synthesis	High (up to several millions)	~ 2
	Intermediate (up to hundreds of thousands)	
Polymers from ionic synthesis	Intermediate (up to hundreds of thousands)	~ 1.01
	Low (up to tens of thousands)	
Polymers from addition synthesis	Intermediate (up to hundreds of thousands)	~ 2
	Low (up to tens of thousands)	
Polymers from controlled radical polymerization	Low (up to tens of thousands)	~ 1.1 to 1.5
	Very low (a few thousand)	
Polyolefins	Intermediate (up to hundreds of thousands)	~ 2 to 200
	High (up to several millions)	
Acrylates	Intermediate (up to hundreds of thousands)	~ 2
	High (up to several millions)	
Small molecule additives	Very low (a few thousand)	1
Pre-polymers	Low (up to tens of thousands)	~ 2 to 10
	Very low (a few thousand)	
Resins	Low (up to tens of thousands)	~ 2 to 10
	Very low (a few thousand)	
Natural biopolymers such as polysaccharides	Intermediate (up to hundreds of thousands)	~ 2 to 10
	High (up to several millions)	
Rubbers	Intermediate (up to hundreds of thousands)	~ 2 to 10
	High (up to several millions)	
Biodegradable polymers	Intermediate (up to hundreds of thousands)	~ 1.1 to 2
	Low (up to tens of thousands)	

[1] Polydispersity is a measure of the distribution of molecular mass of a polymer (polydispersity ratio = Mw/Mn)

Troubleshooting guidelines

- Establish familiarity and good working-order parameters for all equipment
- Keep records of normal operating conditions and chromatograms
- Refer to manufacturer's handbook, call supplier for advice and discuss problems with other users
- Be methodical in solving problems by systematically removing items one at a time and only replacing with known working equipment
- Use a control sample to classify symptoms
- Anticipate problems with good system notes and maintenance records

Ordering information

Organic GPC columns

Description	MW Range (g/mol)	Part Number
PLgel 20 µm MIXED-A, 7.5 x 300 mm	2,000 to 40,000,000	PL1110-6200
PLgel 20 µm MIXED-A LS, 7.5 x 300 mm	2,000 to 40,000,000	PL1110-6200LS*
PLgel 10 µm MIXED-B, 7.5 x 300 mm	500 to 10,000,000	PL1110-6100
PLgel 10 µm MIXED-B LS, 7.5 x 300 mm	500 to 10,000,000	PL1110-6100LS*
PLgel 5 µm MIXED-C, 7.5 x 300 mm	200 to 2,000,000	PL1110-6500
PLgel 5 µm MIXED-D, 7.5 x 300 mm	200 to 400,000	PL1110-6504
PLgel 3 µm MIXED-E, 7.5 x 300 mm	up to 25,000	PL1110-6300
PLgel 3 µm 100Å, 7.5 x 300 mm	up to 5,000	PL1110-6320
PolyPore, 7.5 x 300 mm	200 to 2,000,000	PL1113-6500
ResiPore, 7.5 x 300 mm	up to 500,000	PL1113-6300
MesoPore, 7.5 x 300 mm	up to 25,000	PL1113-6325
OligoPore, 7.5 x 300 mm	up to 3,300	PL1113-6520

** Low shedding for light scattering applications*

Mixed Solvent GPC columns

Description	MW Range (g/mol)	Part Number
PolarGel-M, 7.5 x 300 mm	up to 500,000	PL1117-6800
PolarGel-L, 7.5 x 300 mm	up to 60,000	PL1117-6830

PLgel - see table above

Aqueous GPC/SEC columns

Description	MW Range (g/mol)	Part Number
PL aquagel-OH 60, 15 µm 7.5 x 300 mm	200,000 to 10,000,000	PL1149-6260
PL aquagel-OH 40, 15 µm 7.5 x 300 mm	10,000 to 200,000	PL1149-6240
PL aquagel-OH MIXED-H, 8 µm 7.5 x 300 mm	6,000 to 10,000,000	PL1149-6800
PL aquagel-OH MIXED-M, 8 µm 7.5 x 300 mm	1,000 to 500,000	PL1149-6801
PL aquagel-OH 60, 8 µm 300 x 7.5 mm	200,000 to 10,000,000	PL1149-6860
PL aquagel-OH 50, 8 µm 7.5 x 300 mm	50,000 to 600,000	PL1149-6850
PL aquagel-OH 40, 8 µm 7.5 x 300 mm	10,000 to 200,000	PL1149-6840
PL aquagel-OH 30, 8 µm 7.5 x 300 mm	100 to 60,000	PL1120-6830
PL aquagel-OH 20, 5 µm 7.5 x 300 mm	100 to 20,000	PL1120-6520

Calibration standards

Description	MW Range (g/mol)	Part Number
Agilent EasiVial PEG/PEO 2 mL pre-weighed calibration kit	100 to 1,200,000	PL2080-0201
Agilent EasiVial PEG 2 mL pre-weighed calibration kit	106 to 35,000	PL2070-0201
Agilent PEG-10 polyethylene glycol calibration kit	106 to 20,000	PL2070-0100
Agilent PEO-10 polyethylene oxide calibration kit	20,000 to 1,000,000	PL2080-0101
Agilent SAC-10 pullulan polysaccharide calibration kit	180 to 850,000	PL2090-0100
Agilent PAA-10 polyacrylic acid Na salt calibration kit	1,000 to 1,000,000	PL2140-0100
Agilent EasiVial PS-H 2 mL pre-weighed polystyrene calibration kit	162 to 6,000,000	PL2010-0201
Agilent EasiVial PS-M 2 mL pre-weighed polystyrene calibration kit	162 to 400,000	PL2010-0301
Agilent EasiVial PS-L 2 mL pre-weighed polystyrene calibration kit	162 to 40,000	PL2010-0401
Agilent EasiCal PS-1 pre-prepared polystyrene kit	580 to 7,500,000	PL2010-0501
Agilent EasiCal PS-2 pre-prepared polystyrene kit	580 to 400,000	PL2010-0601
Agilent S-H-10 polystyrene calibration kit	300,000 to 15,000,000	PL2010-0103
Agilent S-H2-10 polystyrene calibration kit	1,000 to 15,000,000	PL2010-0104
Agilent S-M-10 polystyrene calibration kit	580 to 3,000,000	PL2010-0100
Agilent S-M2-10 polystyrene calibration kit	580 to 300,000	PL2010-0102
Agilent S-L-10 polystyrene calibration kit	162 to 20,000	PL2010-0101
Agilent S-L2-10 polystyrene calibration kit	162 to 10,000	PL2010-0105
Agilent M-M-10 polymethylmethacrylate calibration kit	1,000 to 1,500,000	PL2020-0101
Agilent M-L-10 polymethylmethacrylate calibration kit	500 to 50,000	PL2020-0100
Agilent PR-10 protein calibration kit	75 to 2,000,000	PL2150-0100

All the above polymer types are also available as nominal molecular weights

Glossary

Analyte – the substance to be separated by chromatography.

Å – Ångstrom, a unit of length of 10-10 meter (i.e. 0.0000000001 meter), named after Anders Angstrom, a Swedish astronomer and physicist. 1A = 0.1 nanometer.

Back pressure – the resistance (pressure) to flow by column particles when a liquid is pumped through them. Small, more tightly packed particles create higher back pressures than larger particles.

Bonded phase – a stationary phase that is bonded to the particles or to the inside wall of the column.

Chromatogram – the visual display of the results of a chromatography experiment.

Column – hollow steel or glass tube that contains the stationary phase and through which the mobile phase is passed.

Copolymer – a polymer composed of two different repeating units.

Cross-link – a short side chain that links two polymer chains together.

Detector – an instrument that reacts to the presence of an analyte. Detectors can be built in to chromatographs or added as separate modules.

Divinylbenzene (DVB) – a compound having a benzene ring joined to two vinyl groups. DVB reacts with polystyrene (PS) to form a cross-linked copolymer. PS/DVB is the material from which many particles for GPC/SEC columns are made.

Eluent – the mobile phase that enters and leaves a column.

Exclusion limit (of a column) – molecules larger than the exclusion limit cannot enter the pores and so they pass through the column without hindrance.

GPC – gel permeation chromatography.

HPLC – high performance liquid chromatography, in which the mobile phase is forced through a column using a pump.

Hydrophilic – a compound attractive to water molecules.

Immobile phase – see stationary phase.

LC – liquid chromatography, in which the mobile phase is a liquid, aqueous, organic or a supercritical fluid.

Mn – the number average molecular weight.

Mobile phase – the liquid, gas or supercritical fluid that is passed through a chromatography column. The mobile phase is composed of the compounds being analyzed and the solvent that carries them through the column.

Moiety – a characteristic part of a molecule.

Mp – the peak molecular weight, the position of the top of the peak.

Mw – the weight average molecular weight.

Mz – z-average molecular weight.

µm – micrometer (micron) is one millionth of a meter, 10-6 meter, i.e. 0.000001 meter.

nm – nanometer, is 10-9 meter, i.e. 0.000000001 meter.

Oligomer – a polymer made from a few repeat units.

Polydisperse, polydispersity – a polymer is described as polydiperse when it consists of a wide range of molecular sizes. Polydispersity is a measure of the width of the range. Synthetic polymers are polydisperse.

Polyhydroxy – a compound with many hydroxyl (-OH) groups.

Polymer – a large molecule made up of repeating units.

Polystyrene – a polymer made from repeat styrene molecules.

Pore – a hole in a particle.

Refractive index – the ratio of the speed of light in a vacuum to its speed through a compound. The RI of a vacuum is 1, in air it's about 1.0003, in water about 1.33 and in glass about 1.5.

Retention time – the time it takes for a particular analyte to pass through the chromatography system (from the injection valve to the detector).

SEC – size exclusion chromatography.

For further information and useful GPC resources including primers, selection guides, and application compendia, visit **www.agilent.com/chem/GPCresources**

Agilent Ordering Information

For more information on our products and services, visit
www.agilent.com

For technical support, visit
www.agilent.com/chem/techsupport

For your nearest Agilent office or distributor, visit
www.agilent.com/chem/contactus

To place an order, visit
www.agilent.com/chem/store

Agilent offers a complete line of sample preparation products to support LC and LC/MS applications.

The Agilent Bond Elut and Captiva Sample Prep family of products offer the largest choice of formats and widest range of solutions available in the market today.

Learn more at **www.agilent.com/chem/sampleprep**

Published in the UK December 19, 2013
Publication Number: 5991-3080EN
Part Number: 5971-6576

Agilent Technologies