An Introduction
to **Gel Permeation
Chromatography** and **Size
Exclusion Chromatography**

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Contents

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1981 PLgel MIXED columns, PL aquagel columns
MIXED columns improve data quality, with novel chemistries for analysis of water soluble polymers

1984 GPC software
Dedicated software streamlines GPC/SEC calculations

1976 PLgel columns, individual standards and standard kits
Polymer Laboratories founded to develop market leading products for organic GPC/SEC

76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92

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Start here

This guide provides some background to the most common techniques and applications of gel permeation chromatography, also known as size exclusion chromatography (GPC/SEC), for anyone not familiar with the equipment and methods used in this important analytical technique. As this is a basic introduction you do not need to know any chemistry to get something useful from it, though a little knowledge is definitely a good thing. If you want to know more about the science of GPC, there is a reading list in the appendix, and a glossary at the back of this guide to explain some of the most common terms.
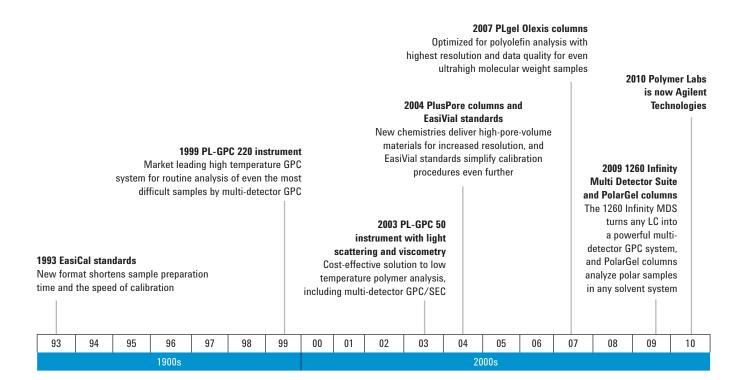
The guide begins with some basic information in Chapter 1 about the history of chromatography and its technologies, but you can skip this and get straight to the chapters on GPC/SEC if you're in a hurry.

A note on names

In this booklet, the terms GPC and SEC are used to describe the same chromatography process with the different acronyms being used by different industries, but generally speaking when analysts discuss GPC and SEC they are referring to the same type of chromatographic analysis. The International Union of Pure and Applied Chemists (IUPAC) prefer the term SEC for experiments of this type, but GPC is still in common use.

Seven things you should know about GPC/SEC

1. Gel permeation chromatography/size exclusion chromatography is a type of high performance liquid chromatography (LC).

2. GPC/SEC can be performed in a wide range of solvents. From non-polar organics to aqueous applications.

3. GPC/SEC uses columns packed with very small, round, porous particles to separate molecules contained in the solvent that is passed through them.

4. GPC/SEC separates molecules on the basis of their size, hence 'size exclusion'.

5. The first GPC/SEC columns were packed with materials referred to as gels, hence 'gel permeation'.

6. GPC/SEC is used to determine the molecular weight distributions of polymers.

7. The particles in the columns are made from polymers that have been cross-linked to make them insoluble, or inorganic materials, such as spherical silicas.

2007 PLgel Olexis columns
Optimized for polyolefin analysis with highest resolution and data quality for even ultrahigh molecular weight samples

2010 Polymer Labs is now Agilent Technologies

2004 PlusPore columns and EasiVial standards
New chemistries deliver high-pore-volume materials for increased resolution, and EasiVial standards simplify calibration procedures even further

1999 PL-GPC 220 instrument
Market leading high temperature GPC system for routine analysis of even the most difficult samples by multi-detector GPC

2009 1260 Infinity Multi Detector Suite and PolarGel columns
The 1260 Infinity MDS turns any LC into a powerful multi-detector GPC system, and PolarGel columns analyze polar samples in any solvent system

2003 PL-GPC 50 instrument with light scattering and viscometry
Cost-effective solution to low temperature polymer analysis, including multi-detector GPC/SEC

1993 EasiCal standards
New format shortens sample preparation time and the speed of calibration

93	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10
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Chapter 1 – What is chromatography?

The invention of chromatography, its techniques and the place of GPC/SEC within the chromatography family

Chromatography is a separation method used for chemical analysis invented by the Russian, Mikhail Tsvet, in 1901. Tsvet ground up plant leaves in solvents such as ether and ethanol that dissolved the chlorophyll and carotenoid pigments in the leaves. He poured the resulting solution into a glass column filled with solid calcium carbonate, using gravity to draw the solution down the column. Colored bands were formed as the pigments in the solution moved through the column at different speeds due to greater or lesser degrees of interaction with the calcium carbonate, revealing that the extract was made up of different components. Tsvet was able to collect these different colored molecules into separate containers as they dripped or, 'eluted', from the bottom of the column.

This simple experiment demonstrated the great potential of chromatography in separating mixtures of molecules. Today, samples can be gases, liquids or solids, in simple mixtures or in complex blends of widely differing chemicals. The solvent can also be a gas or liquid, depending on the type of chromatography.

Chromatography systems employ a column, capillary or some other container that is filled with a mobile and a stationary phase, which can be solid, liquid or gas. The stationary phase remains in position and does not move during the analysis, whereas the mobile phase moves through the container. In Tsvet's experiment, the stationary phase was the calcium carbonate and the mobile phase was the organic solvent.

The separation occurs because molecules partition between the two phases, and the more association the molecule has with the stationary phase, the longer it takes to leave the container. All the different forms of chromatography that scientists use today describe the use of different mobile and stationary phases. You will come across some examples later.

Since the early days, a great deal of work has been done to find the best method of detecting the components as they exit the container holding the mobile and stationary phases, usually based on their chemical or physical properties. These include color, viscosity, the way the molecules behave when light is shone through them, or whether they carry an electric charge. Not surprisingly, chromatography equipment and techniques have also been refined over the years. For example, Tsvet relied on gravity to move his extracts through the column, but now high-pressure pumps or compressed gases are often used.

Chromatography is now accepted as probably the most powerful and versatile analytical technique available because it can separate mixtures in a single step, and measure the amount of every component and their relative proportions.
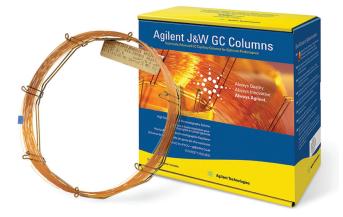
Chromatography instruments are widely used in academia and industry for research and development of new compounds. The instruments can be complex and expensive, or simple and inexpensive, so much so that practical chromatography can be done in school. One of the most common chromatography demonstrations in schools is to separate plant pigments – Mikhail Tsvet would be pleased.

Types of chromatography

There are many forms of chromatography, but two are very common and well known to all analytical scientists and serve to illustrate the diversity of analytical techniques. These are gas chromatography (GC) and liquid chromatography (LC). GPC/SEC is a form of LC. These techniques are described in brief below.

Gas chromatography

In GC, the stationary phase is (typically) polydimethylsiloxane (PDMS) applied to the inner surface/walls of a very narrow capillary tube. A gas such as helium is used as the mobile phase. Volatile samples, or samples volatilized by heating, are introduced into the gas phase and flow through the column, during which the components of the sample have time to interact with the stationary phase, mainly through physical interactions and adsorption. As a result, molecular components of the sample are separated based on their degree of affinity for the stationary phase (of which there are a very wide variety which are

application dependent). Components exiting the column are then "detected", typically using a flame ionization detector or a mass spectrometer. GC is the preferred technique for analyzing volatile samples that contain molecules of differing chemistries.
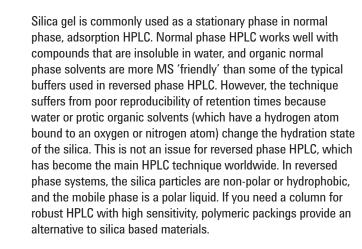

Agilent J&W offers the largest selection of the highest quality GC columns available today

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

High performance liquid chromatography

HPLC is a technique that employs a liquid mobile phase. Stationary phases are typically chemically modified inorganic silicas or polymeric beads packed into a column. In HPLC, the separation mechanism that partitions components of a sample between the two phases can take many forms, depending on the

nature of the samples and the phases employed. For example, in one of the most common types of HPLC, the sample components interact with the stationary packing material based on how hydrophobic, or "greasy", the components are. A mobile phase that can dissolve these hydrophobic components is then passed through the column. Those components that are only somewhat greasy will easily come off, while those that are more greasy will come off the column much later.

Agilent's HPLC column range includes Poroshell, ZORBAX, Polaris, PLRP-S, and more

Silica gel is commonly used as a stationary phase in normal phase, adsorption HPLC. Normal phase HPLC works well with compounds that are insoluble in water, and organic normal phase solvents are more MS 'friendly' than some of the typical buffers used in reversed phase HPLC. However, the technique suffers from poor reproducibility of retention times because water or protic organic solvents (which have a hydrogen atom bound to an oxygen or nitrogen atom) change the hydration state of the silica. This is not an issue for reversed phase HPLC, which has become the main HPLC technique worldwide. In reversed phase systems, the silica particles are non-polar or hydrophobic, and the mobile phase is a polar liquid. If you need a column for robust HPLC with high sensitivity, polymeric packings provide an alternative to silica based materials.

Gel permeation chromatography/size exclusion chromatography

As we have seen, GPC/SEC is a type of LC and so solid stationary and liquid mobile phases are again used. However, the separation mechanism here relies solely on the size of the polymer molecules in solution, rather than any chemical interactions between particles and the stationary phase.

Chapter 2 – GPC/SEC overview

GPC/SEC instruments produce information on polymer molecular weight distributions

GPC/SEC employs a stagnant liquid present in the pores of beads as the stationary phase, and a flowing liquid as the mobile phase. The mobile phase can therefore 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 physical characteristics that make them attractive to industry, and to us as consumers. Beneficial physical properties, include hardness, thermal and electrical insulation, optical properties, and resistance to chemicals. These parameters are influenced by a polymer's attributes, such as chemistry, molecular structure and shape, molecular weight and the presence of branching. Furthermore, these parameters can be characterized by measuring some basic attributes. The physical attributes can either be directly assessed by examination of the finished product or be predicted from an understanding of the polymer molecules.

So what exactly are polymers? Polymers are molecules 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. Does this matter? Yes it does!

Size matters

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 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. Clearly, the example of the 'liquid – wax – solid' we mentioned above is an extreme case, but you don't need much of a difference in the distribution of polymer chain lengths to drastically alter its physical properties.

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!
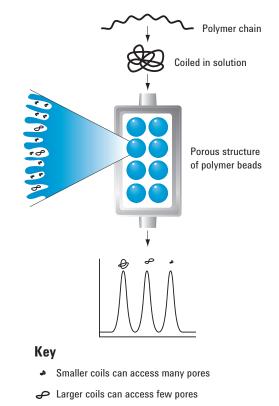
How does GPC/SEC work

A GPC/SEC instrument consists of a pump to push the solvent through the instrument, an injection port to introduce the test 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.

The polymer sample is first 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 coil 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. Conversely, 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. Imagine you are walking with a child through the toy section of a department store. You want to get straight to the car park, but your small companion wanders off to sample all the delights on offer, so you will reach the exit straight away while junior takes his time to get there, pausing to investigate all the toys on display. It is the same with GPC/SEC – the larger bodies get to the exit first.

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.

Key

🌀 Smaller coils can access many pores

🌀 Larger coils can access few pores

🌀 Very large coils access very few pores

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

As the components exit the column they are detected in various ways, 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 you have a constant flow rate. 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, because the molecules have been retained on the column.

The data that produced the chromatogram is then compared to a calibration that shows the elution behavior of a series of polymers for which the molecular weight is known. This allows the molecular weight distribution of the sample to be calculated, providing important information for polymer chemists because they can use the distribution to predict how the polymer will perform.

One important thing to bear in mind about GPC/SEC – the separation is based on size, not chemistry. These techniques give information regarding the size of polymer molecules in solution that are converted into molecular weights through the use of a calibration. They don't tell us anything about the chemistry of the sample, or even if the sample has components of different chemistries. GPC/SEC is purely a physical partitioning of the sample on the basis of size.

Who uses GPC/SEC, what for and why

GPC/SEC has two main uses – to characterize polymers and 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, a property all synthetic polymers have. Furthermore, 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.

To avoid damage to fragile biological compounds during chromatography, biologists and biochemists typically use low pump pressures and columns packed with a gel, such as polyacrylamide, dextran or agarose. The advantage of the technique for these scientists is that the biological activity of the compounds is not destroyed and so the fractions that come out of the column can be used in other experiments, though the technique is not very efficient. On the other hand, 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 combined with additional methods that further separate molecules by other characteristics, such as their acidity, basicity, charge or affinity.

Now that we know how GPC/SEC works, here's some detail on how we calculate molecular weights.

Calibrations

As we have seen, to determine the molecular weights of the components of a polymer sample, a calibration with standard polymers of known 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 molecular weights, and as kits and 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 we use to set up 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 (we'll come on to polydispersity soon).

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

You can see that the molecular weight is determined from the calibration curve by simply noting the retention time (RT) of the sample, and reading the molecular weight on the vertical
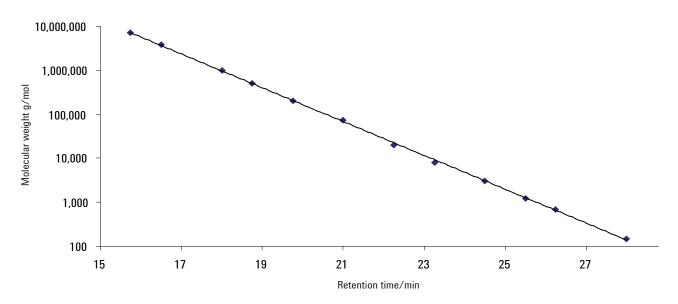
Figure 2. A calibration graph used to determine the molecular weight of a polymer from its retention time

axis that corresponds to this time. To generate a molecular weight distribution, we cut the peak into a number of equally spaced 'slices'. We measure the abundance of each slice from the peak height or area at that point, and the molecular weight of the slice from the calibration. We then perform some summations to get our averages – more on this below.

It is also possible to measure sizes directly by using some form of detector that reacts directly to the molecular weight of the components as they leave the column. These detectors rely on the physical properties of the polymer, such as their ability to scatter light, or their viscosity.

Calculations in GPC/SEC

In polymers, as we have already seen, 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, we have to count how many particles there are at every weight in the distribution, and from these counts, calculate an average figure that describes the whole sample. Figure 3 shows the typical position of molecular weight average.

The commonly calculated average is actually called the number average molecular weight, abbreviated to Mn. Looking at the distribution in Figure 3 we can see that the Mn value marks the value at which there are equal numbers of molecules on each side, at higher and lower molecular weight. The value of Mn influences the thermodynamic properties of the molecule. There are several other ways of describing molecular weight average, including weight average molecular weight (Mw). Mw is defined as the value
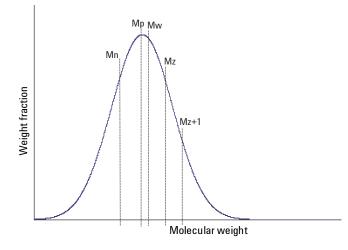

Figure 3. The average molecular weights of a mono-modal polymer – in this case the distribution is nearly symmetrical

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 material's range of molecular mass. The broader the molecular weight distribution, the larger the PDI.

However, obtaining all these numbers is not as easy as it seems. On their own, standard GPC/SEC detectors cannot count the number of molecules that elute from the column, so these weight averages cannot be measured directly. Nonetheless, we can measure the concentration of molecules on a weight/volume basis, and calculate the weight averages from the concentration using a concentration detector, such as one that measures the 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 we can see 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 a graph's axis when very large numbers are involved, and 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.

You can see from this example that knowledge of the molecular weight distribution of a polymer 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 whether the end product of a production run will be able to meet the industrial specification required. In this way, expensive mistakes can be avoided.
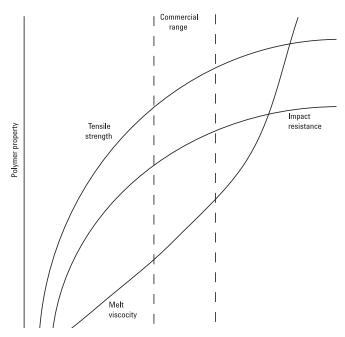

Figure 4. The effects of molecular weight on the properties of polymers

10

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 dispersity of the polymer. Dispersity 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 we come across are those found in nature, such as proteins and nucleic acids.

- Narrow distribution polymers are synthetic compounds where we try and make all the chains as close in molecular weight as possible. The Mw/Mn polydispersity ratio is less than 1.2, a subjective but handy definition of a polymer with a narrow distribution. Examples include polymers used as standards for calibrations – here we want to determine the position of the top of the peak as accurately as we can,

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 have a medium width distribution, with an Mw/Mn ratio between 1.2 and 3, because many of the synthetic 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 and this is where it gets interesting. 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 – you have to look at the molecular weight distribution to see that.

The molecular weight averages and the ratio of Mw to Mn are thus very useful in revealing the width of a polymer distribution, but you need to look at the molecular weight distribution plot to assess the shape of the distribution. However, together with Mw, polydispersity values provide very useful information on the character of a polymer, but they is no substitute for a close examination of the molecular weight distribution plot to get the whole story.

Agilent offers a portfolio of standards for GPC/SEC

Figure 5 shows representative molecular weight distributions for polymers of differing polydispersities. A narrow distribution consists of a single, sharp peak, with an Mw/Mn of <1.2. A medium distribution has a wider range in the size of polymer chains with an Mw/Mn 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.
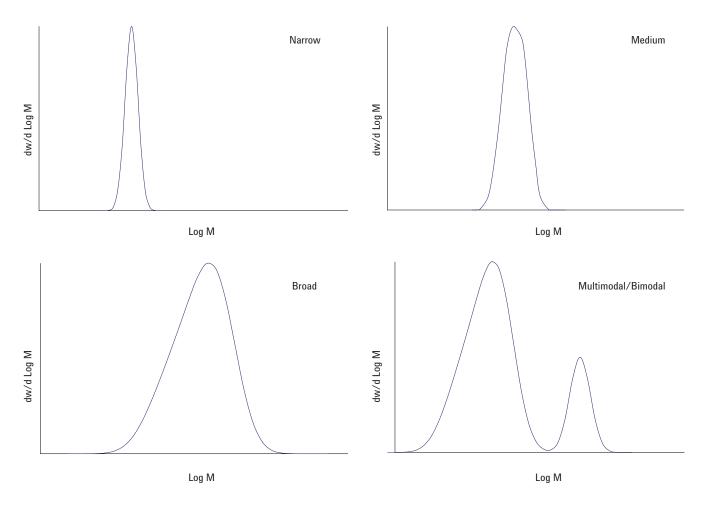

Figure 5. *Different polymer molecular weight distributions*

Chapter 3 – GPC/SEC in practice

The component parts of a GPC/SEC system and how they work together

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). We're going to look at each of these functions in turn to try to build a picture of what a GPC/SEC system does 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, as 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.

Ovens

GPC/SEC is usually carried out at room temperature, but some instruments have heated and thermostatically controlled ovens in which the columns and detectors are placed. Higher temperatures, up to 220 °C, are necessary for some solvents that have much higher viscosities, such as trichlorobenzene or chloronaphthalene, than more usual organic solvents like tetrahydrofuran, chloroform or toluene. Operating the instrument at high temperatures reduces viscosity and hence column back pressure, with a corresponding increase in efficiency. Some samples are also insoluble at lower temperatures; in these cases high temperature is required to maintain the solubility of the sample during the analysis.
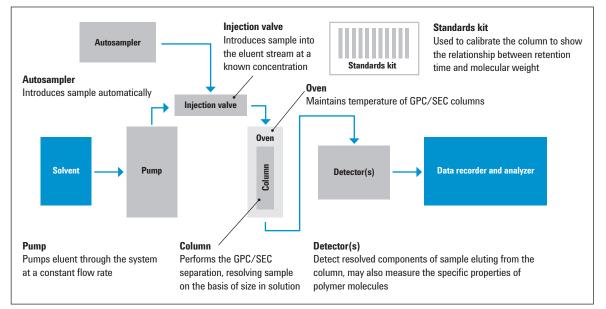

Autosampler
Introduces sample automatically

Injection valve
Introduces sample into the eluent stream at a known concentration

Standards kit
Used to calibrate the column to show the relationship between retention time and molecular weight

Oven
Maintains temperature of GPC/SEC columns

Pump
Pumps eluent through the system at a constant flow rate

Column
Performs the GPC/SEC separation, resolving sample on the basis of size in solution

Detector(s)
Detect resolved components of sample eluting from the column, may also measure the specific properties of polymer molecules

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 before being put through the chromatograph, which may take up to 12-24 hours.

Where possible, the eluent used to prepare the samples should be the same as the solvent running through the system. Sample concentration employed during analysis is dependent on the molecular weight and the viscosity of the sample under investigation. Table 1 gives some common sample concentrations and corresponding injection volumes for analytical GPC/SEC.

Table 1. *Different sample concentrations for different polymer molecular weights*

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

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.

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 20-200 µL, see Table 2. It is important that the injector does not disturb the flow of the mobile phase.

Columns and column sets

Separation of the sample takes place inside the column, a hollow tube tightly packed with extremely small porous beads, typically polymer or silica, designed to have pores of well defined size. The columns vary in length from 50 mm to 300 mm, and internal diameters of 4.6 to 25 mm, depending on their intended use. For example, the smaller columns (7.5 x 50 mm id) are used as guard columns, mid sizes (4.6 x 250 mm or 7.5 x 300 mm) for analytical work and the largest (25 x 300 mm) for preparative methods. The type of bead 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 narrow particle sizes between 3 to 20 µm. The pore sizes range from 50 to 1,000,000Å. It's a hangover from the early days of GPC/SEC that the pore 'size' is the length of the polystyrene molecule in its natural, undissolved state, that when dissolved in tetrahydrofuran and coiled in a sphere would only just fit in the pore – it is not the diameter of the pore. In HPLC, however, the pore size really is the pore size!

Columns are packed with different sized particles with different sized pores primarily for different molecular weight ranges. The reason for this specificity is that the nature of the sample and solvent determines which column configuration will provide the best analytical result, which is 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, as shown in Table 2. 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.

Table 2. *GPC/SEC column applications, benefits and formats*

Application requirement	Recommended Agilent column	Benefit	Column format	Organic or Aqueous (O or A)
Routine analysis of synthetic polymers	PlusPore, PLgel	Wide pore size distribution covers extended MW range	Analytical high resolution	O
To remove background 'noise' when using light scattering detectors	PLgel LS	Absence of micro particles gives cleaner light scattering data	Analytical light scattering	O
Save solvent costs	PLgel MiniMIX	Narrow bore reduces solvent consumption	Analytical narrow bore	O
Fractionation of polymers	PLgel	Large column internal diameters allow large injection volumes	Preparative	O
Application specific, e.g. polyester/ polyamides in hexafluoroisopropanol	PL HFIPgel	Tailored particle chemistry	Analytical speciality	O
Polyolefins	PLgel Olexis			O
Sample clean-up for pesticides	EnviroPrep			O
Routine analysis of naturally occuring polymers	PL aquagel-OH	Very hydrophilic particle exterior	Analytical and preparative	A
Screening molecular weight distribution for trend analysis	PL Rapide	Reduced column length and ability to cope with high solvent flow rate	Analytical high speed	O & A
Polar, non-water soluable polymers	PolarGel	Intermediate polarity surface on particles	Analytical	O & A
Biomolecules	Bio SEC	Silica particle for high resolution	Analytical	A
To prevent contamination by non-volatile residues	Available for all analytical columns	Same particle chemistry and particle size	Guard	O & A

A more detailed guide to column choice is given in the appendix

Pumps

The pump takes the solvent and delivers it to the rest of the system 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 expensive items of equipment because they have to be made from inert materials such as stainless steel, titanium and ceramics, which do not react with the solvents used in GPC/SEC. They must also withstand very high pressures.

The Agilent PL-GPC 50 Integrated GPC/SEC System with autosampler on the left, display on the right and solvent pump, column oven and detector in the housing in the middle

15

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.

The Agilent PL-GPC 220 Integrated GPC/SEC System is an extremely powerful system, designed to run almost all polymer, solvent and temperature combinations

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, shown on page 15, 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.

Chapter 4 – GPC/SEC in action; real world applications

Some examples that demonstrate the value of gel permeation chromatography and size exclusion chromatography

Gum arabic, good and bad

Gum arabic is a polysaccharide widely used in the food industry as a viscosity modifier or gelling agent. The physical properties and processibility of these water soluble polymers are related to their molecular weight distributions, which can be determined by SEC using two Agilent PL aquagel-OH columns. A comparison of the molecular weight distributions of a 'good' and 'bad' sample (Figure 7) shows definite differences between the two batches of gum arabic, the 'bad' sample having considerably more high molecular weight material, which could make it unusable for an industrial process.
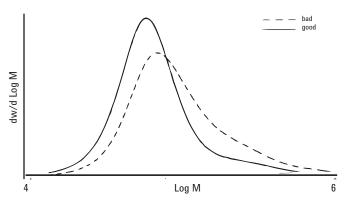

Figure 7. *The difference between batches of gum arabic labelled 'good' and 'bad' can be clearly seen by overlaying their molecular weight distributions*

Fingerprinting nail varnish

Commercial nail varnishes are complex formulations typically containing six components:

- Cellulose film formers add gloss to the nail
- Resins improve the gloss and ensure adhesion
- Plasticizers give flexibility to the cellulose component
- Pigments add color
- Thixotropic agents maintain the pigments in suspension
- A mixture of solvents carry the components to the point of application

The properties of the cellulose and resin determine the hardness and durability of the formulation, strongly influencing the quality of the final finish. Two of the most common materials used in nail polishes are toluene sulfonamide formaldehyde resin (TSF) and nitrocellulose.

The components work in synergy to give the desired characteristics of the nail polish; the soft, dull looking TSF resin gives good adhesion to the nail plate while the nitrocellulose strengthens the TSF and makes the coating hard and shiny. For both these materials, molecular weight is the key parameter controlling performance in the final application (Figures 8a and 8b).
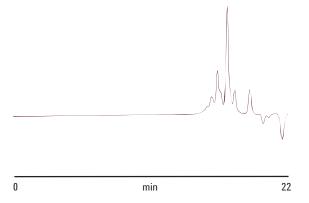

Figure 8a. *Agilent OligoPore columns separate the components of nail varnish, revealing the individual oligomers and their relative proportions*
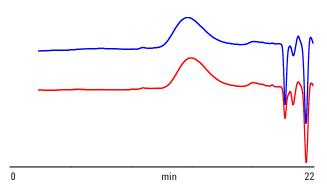
Figure 8b. This overlay of two batches of nitrocellulose run on Agilent MesoPore columns shows that they are very similar, indicating that they will be equally effective if they are used as components in a nail varnish – this helps manufacturers ensure that different batches of nail varnish perform in the same way

Modifying PVC

Poly(vinyl chloride) is a thermoplastic widely used in many products and industries. Its physical and mechanical characteristics make it ideal for many different uses, for example in the toiletry, food, water, car and construction industries.

Unplasticized PVC has a high melt viscosity leading to some difficulties in processing. The finished product is also too brittle for many applications. In order to overcome these problems, it is routine to include additives as impact modifiers or processing aids, to ensure more uniform flow and hence improve surface finish. The properties of the final material are dependent on the molecular weight distribution of the PVC and the type and level of the added plasticizers.

The analysis of the compounded material is, therefore, of primary importance, and GPC/SEC is the ideal analytical tool for its characterization.

Three different grades of PVC tubing containing different plasticizers were analyzed (Figure 9), employing a column set comprising 3 x Agilent PLgel 5 µm MIXED-C columns (300 x 7.5 mm). All three samples displayed a broad peak at the same retention time that was due to the PVC (approx 17 minutes), but also displayed other peaks with varying retention times which were attributed to the different plasticizers. Sample 1 contained an aliphatic plasticizer and sample 2 an aromatic one. Sample 3 contained both. The common peak eluting at about 29 minutes was due to toluene which was included in the samples for flow rate correction.
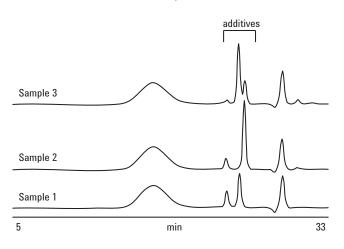

Figure 9. Chromatograms of samples of PVC showing the presence of different additives in the low molecular weight region

Chapter 5 – FAQs

Some common questions from GPC/SEC users

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 PlusPore columns are packed with beads of polystyrene/divinylbenzene copolymer, 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 Agilent 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 µm 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.

Appendix

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	Answer	Recommendation	Comments
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.*	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	Answer	Recommendation	Comments
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.*	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 offers the best possible oligomer separation, PLgel also works 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

21

Setting up the GPC/SEC system

Question	Answer	Recommendation	Comments
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.*	Depends on the particle size of the columns	Particle size 20 µm use 4 columns 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	Increased number of columns required for large particle sizes to make up for low efficiencies
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. Injection volume also varies dependent on column id. 4.6 mm id columns will require a lower injection volume than 7.5 mm id columns, and 25 mm id columns will require a higher injection volume.*	Depends on the particle size of the columns	Particle size 20 µm use 200 µL injection 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	Smaller particle sizes require smaller loops to minimize band broadening

What standards should I use?

Standards shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
5. What is the eluent? *Standards are polymers, so the choice of standard mainly reflects solubility in the chosen eluents.*	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
6. What format of standards are recommended? *Different formats of standards are available depending on customer preference.*	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 polydispersity[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)

Ordering Information

Table 3. *Ordering information for columns for organic solvents*

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

* Low shedding for light scattering applications

Table 4. *Ordering information for columns for mixed solvents*

Mixed Solvent GPC columns		
Description	**MW Range (g/mol)**	**Part Number**
PolarGel-M, 300 x 7.5 mm	up to 500,000	PL1117-6800
PolarGel-L, 300 x 7.5 mm	up to 60,000	PL1117-6830
PLgel - see Table 3		

Table 5. *Ordering information for columns for aqueous solvents*

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

For a full list of GPC/SEC columns, go to **www.agilent.com/chem/gpcsec**

Table 6. *Ordering information calibration standards*

Calibration standards		
Description	**MW Range (g/mol)**	**Part Number**
Agilent EasiVial PEG/PEO 2 mL pre-weighed calibration kit	106 to 1,500,000	PL2080-0201
Agilent EasiVial PEG 2 mL pre-weighed calibration kit	106 to 30,000	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 PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 7,000,000	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 500,000	PL2010-0301
Agilent PS-L EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 50,000	PL2010-0401
Agilent EasiCal PS-1 pre-prepared polystyrene kit	580 to 7,000,000	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

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

For a full list of calibration standards, go to **www.agilent.com/chem/gpcsec**

Agilent solutions for GPC/SEC

Agilent manufactures a comprehensive range of GPC/SEC instruments and consumables, creating superior solutions that address real world problems

Multi-detector GPC

The versatile Agilent 1260 Infinity Multi-Detector Suite (MDS) contains any combination of refractive index, light scattering and viscometry detectors, all individually heated to 60 °C for increased baseline stability and quality of results. Just connect the 1260 Infinity MDS to an LC system capable of isocratic eluent delivery to create a powerful and versatile GPC system. Data collection is managed by a dedicated, integrated control module, including analog inputs allowing external detector and 1260 Infinity MDS data collection into the same data file, for economical use of existing detectors. You can choose manual or software control, or both, to suit your preference.

GPC under ambient conditions

The Agilent PL-GPC 50 Integrated GPC/SEC System is a standalone GPC/SEC instrument containing all the components necessary for the analysis of a wide range of polymers. With pump, injection valve, column oven and optional degasser, as well as any combination of refractive index, light scattering and viscometry detectors, the PL-GPC 50 is an ideal choice when you are starting out in GPC or want the convenience of a single solution.

The column oven operates up to 50 °C, reducing column pressures in viscous solvents to improve column lifetime and chromatographic quality. Software control uses a straightforward and intuitive interface, simple to use and suitable for users with the minimum knowledge of chromatography.

Easy and reliable polymer characterization up to 100 °C

The Agilent 1260 Infinity GPC/SEC Analysis System uses a precise isocratic solvent delivery system to provide the constant, stable flow rate that is essential to maintain high resolution of GPC/SEC columns. The high flow precision and the excellent temperature stability of the thermostatted column compartment ensure highest accuracy and precision of molecular weight determinations. Column temperature stability is guaranteed from 10 °C below ambient to 80 °C, or alternatively to 100 °C.

High temperature GPC

The Agilent PL-GPC 220 Integrated GPC/SEC System is ideal for even the most demanding GPC experiments. With heating of columns, injection valve and detectors up to 220 °C, the PL-GPC 220 is the most advanced GPC system, permitting the analysis of virtually any polymer in any solvent.

Flexible detector options include any combination of refractive index, light scattering and viscometry detectors. You can easily upgrade by adding extra detectors at any time. For difficult applications where elevated temperature is required at all times to maintain sample solubility, the PL-GPC 220 is the best and most reliable choice.

Automating GPC/SEC

Agilent autosamplers are simple yet powerful tools for automating GPC systems. Multiple autosamplers are availble from the 1200 Infinity Series as well as the PL-GPC 50 autosampler.

A choice of autosampler trays gives flexibility in choice of vial size, with full integration and automation using the Agilent GPC/SEC software.

The Agilent 1260 Infinity GPC/SEC Analysis System

Glossary and abbreviations

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 Ångstrom, a Swedish astronomer and physicist. 1Å = 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.

Shear degradation – in which a combination of mismatched pump pressure, sample size, particle size and mobile phase cause the sample molecules to break into smaller pieces in the column.

Solute – the sample dissolved in the eluent.

Stationary phase – the fixed packing of the column that does not move.

Supercritical fluid – a substance kept at a critical temperature and pressure above which it behaves like a gas and liquid. Supercritical fluids can diffuse through solids like a gas and dissolve materials like a liquid. The most common supercritical fluid used in chromatography is carbon dioxide.

Ultrasonicator (sonicator) – an instrument that produces very high frequency sound; used for dissolving, mixing, cell breakdown, emulsification and degassing of liquids.

Viscosity – a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress.

Suggestions for further reading

Dong, M (2006) *Modern HPLC for Practicing Scientists.*
John Wiley & Sons, Hoboken, NJ, U.S.A.

Houlgate, P & Lee, E (1999) *Guide to Improving Analytical Quality in Chemistry.* LGC, Teddington, UK.

Lewis, GF (1985) *Analytical Chemistry, An Introduction –
2nd Ed.* Macmillan, London, UK.

Mori, S & Barth, H (1999) *Size Exclusion Chromatography.* Springer Verlag, Berlin, Germany.

Striegel, A (2004) *Multiple Detection in Size-Exclusion Chromatography.* American Chemical Society, Washington, DC, U.S.A.

Wu, C-S (2003) *Handbook of Size Exclusion Chromatography and Related Techniques.* Marcel Dekker, New York, NY, U.S.A.

Striegel, AM, Yau, WW, Kirkland, JJ & Bly, DD (2009) *Modern Size-Exclusion Chromatography.* John Wiley & Sons, Chichester, UK.

GPC/SEC publication	Publication number
Application compendia	
Analysis of polymers by GPC/SEC - energy & chemicals applications	5991-2517EN
Analysis of polymers by GPC/SEC - food applications	5991-2029EN
Analysis of polymers by GPC/SEC - pharmaceutical applications	5991-2519EN
Excipient analysis by GPC/SEC and other LC techniques	5990-7771EN
Biodegradable polymers - analysis of biodegradable polymers by GPC/SEC	5990-6920EN
Analysis of engineering polymers by GPC/SEC	5990-6970EN
Analysis of elastomers by GPC/SEC	5990-6866EN
Analysis of polyolefins by GPC/SEC	5990-6971EN
Low molecular weight resins - Analysis of low molecular weight resins and prepolymers by GPC/SEC	5990-6845EN
Primers	
Calibrating GPC/SEC columns - a guide to best practice	5991-2720EN
Selection guide	
Quick guide for selecting columns and standards for gel permeation chromatography and size exclusion chromatography	5990-6868EN
Product guides	
Organic GPC/SEC columns	5990-7994EN
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GPC/SEC standards	5990-7996EN

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Agilent and Polymer Laboratories

Agilent Technologies in Shropshire, UK, formerly Polymer Laboratories, was founded in 1976 from the Polymer Research Group at Loughborough University. Our objectives are 'to develop techniques and instrumentation for the characterization of polymer systems, and to develop high technology polymer products for application in the fields of chromatography, diagnostics and pharmaceuticals'.

The Separation Science group offers complete solution approaches for aqueous and organic polymer characterization by size exclusion chromatography (GPC/SEC). We develop and manufacture fully integrated GPC systems, as well as data handling, chromatography columns and polymer standards. We also manufacture a range of ligand-exchange columns for the analysis of carbohydrates, organic acids and alcohols by HPLC. Over a decade ago, the Separation Science group expanded into the life science arena with chromatography products for the analysis and purification of biomolecules. In addition, we produce a range of instruments and consumables for flash purification during drug discovery.

The Microparticles for Life Sciences group develops and manufactures particles for clinical assays and tests ranging from personal pregnancy tests through to HIV assays, in close partnership with major clinical companies around the globe. The wide repertoire of technologies spans latex, magnetic, highly colored and functionalized particles for these applications.
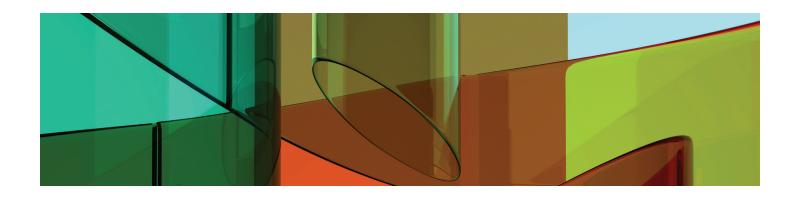
Our expertise in resins for therapeutic peptide and oligonucleotide synthesis has expanded the Pharmaceutical Drug Discovery business beyond chromatography, to focus on resins for drug development using medicinal and combinatorial chemistry — the fastest expanding area in science today.

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To learn more about calibrating your GPC columns, refer to the primer *Calibrating GPC Columns - A Guide to Best Practice* (5991-2720EN).

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