

GPC - Gel Permeation Chromatography
aka Size Exclusion Chromatography- SEC

Wendy Gavin

Biomolecular Characterization Laboratory

Version 1

May 2016

Table of Contents

1. GPC Introduction.....	Page 3
2. How GPC works.....	Page 5
3. GPC Systems.....	Page 7
4. GPC/SEC Separations – Theory and System Considerations...	Page 9
5. GPC Reports.....	Page 10
6. Calibrations of GPC systems.....	Page 14
7. GPC preparation.....	Page 16
8. Alliance System.....	Page 17
9. GPC columns.....	Page 18

1. GPC Introduction

Gel permeation chromatography (GPC) is one of the most powerful and versatile analytical techniques available for understanding and predicting polymer performance. It is the most convenient technique for characterizing the complete molecular weight distribution of a polymer.

Why is GPC important?

GPC can determine several important parameters. These include number average molecular weight (M_n), weight average molecular weight (M_w), Z weight average molecular weight (M_z), and the most fundamental characteristic of a polymer its molecular weight distribution (PDI)

These values are important, since they affect many of the characteristic physical properties of a polymer. Subtle batch-to-batch differences in these measurable values can cause significant differences in the end-use properties of a polymer. Some of these properties include:

Tensile strength	Adhesive strength	Hardness
Elastomer relaxation	Adhesive tack	
Stress-crack resistance		
Brittleness	Elastic modulus	Cure time
Flex life	Melt viscosity	
Impact strength	Tear Strength	
Toughness	Softening temperature	

Polymer Molecular Weight Distributions

Samples of synthetic polymers *always* contain polymer chains with a range of chain lengths

One way to describe the length of the polymer chains is in terms of an **average** molecular weight, i.e the average of all the chain lengths in the sample

HOWEVER....

Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production

In polymer science it is the molecular weight **distribution** that is important

Molecular Weight Averages by GPC

Number average M_n

M_n can be correlative with polymer colligative properties, e.g. freezing point depression

Weight average M_w

M_w may be correlated with properties such as melt viscosity

Z average M_z

M_z may be correlated with properties such as toughness

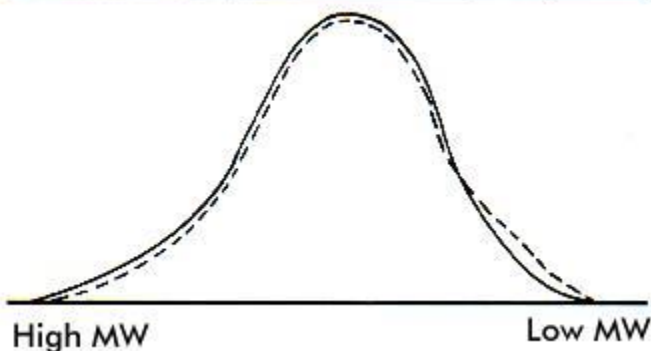
Polydispersity, $d = \frac{M_w}{M_n}$

Polydispersity characterises the shape of the distribution

Telling good from bad

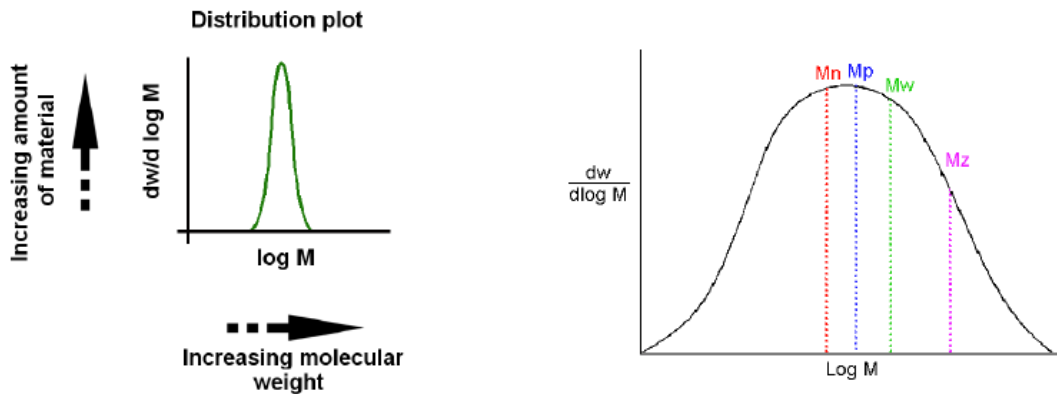
Two samples of the same polymer resin can have identical tensile strengths and melt viscosities, and yet differ markedly in their ability to be fabricated into usable, durable products. These differences can be attributed to subtle, yet significant variations in the molecular weight distributions of the two resin samples. Such differences, if undetected, can cause serious product defects.

Molecular Weight Distribution (MWD)



Though they are subtle, differences such as those shown in the molecular-weight distributions, could cause marked variations in the performance of the polymer.

Defining the Molecular Weight Distribution



A molecular weight distribution can be defined by a series of average values
Except M_p , these are various moments of the average of the molecular weights
of the distribution

M_p is the molecular weight of the peak maxima

For any polydisperse peak:

$$M_n < M_w < M_z < M_{z+1}$$

2. How GPC works

GPC separates molecules in solution by their "effective size in solution." To prepare a sample for GPC analysis the resin is first dissolved in an appropriate solvent.

Polymer Molecules in Solution

- GPC is based on the behaviour of polymer molecules in solution
- In the solid state polymers can be considered like spaghetti – a confusing mass of intertwined chains
- In solution, polymer molecules are discrete entities
- Due to entropic effects all but the most rigid of polymer chains curls up in solution to form a ball like shape



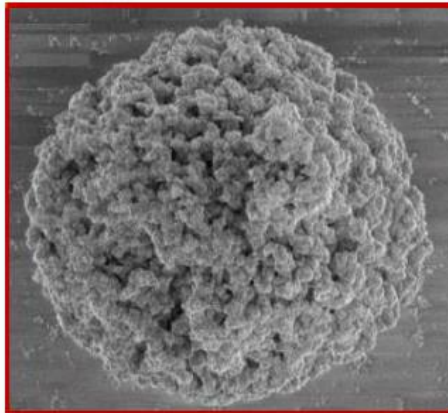
Dissolution



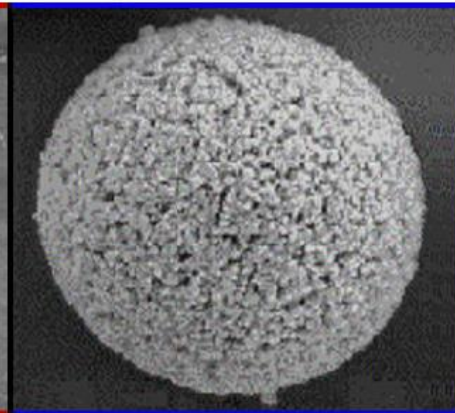
GPC Column Packings

- GPC columns are packing with cross-linked, insoluble beads, typically copolymers of styrene and divinyl benzene for organic GPC
- These beads have a rigid pore structure that remains intact in the presence of solvent

PLgel 10 μm 10^6A

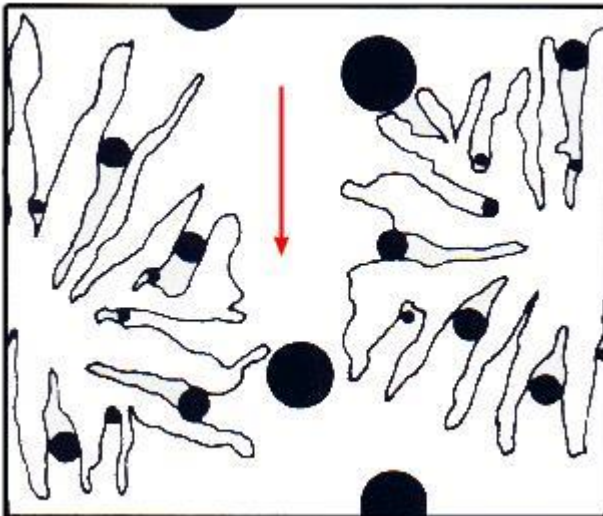


PLgel 10 μm 10^3A



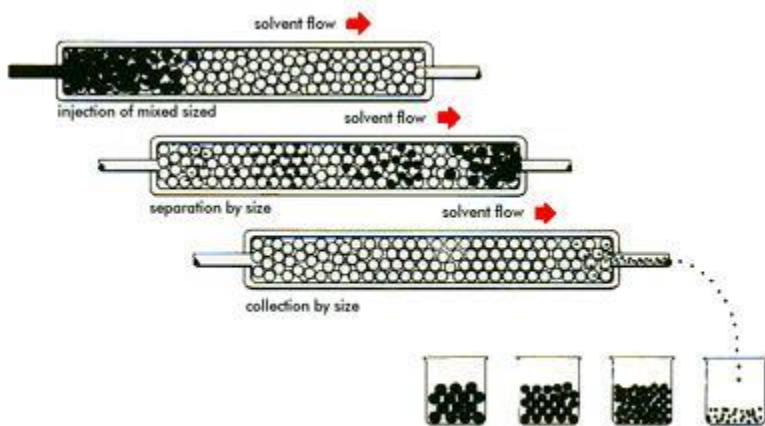
Inside the gel permeation chromatograph, the dissolved resin is injected into a continually flowing stream of solvent (mobile phase). The mobile phase flows through millions of highly porous, rigid particles (stationary phase) tightly packed together in a column. The pore sizes of these particles are controlled and available in a range of sizes.

Cross sectional view of porous particle



The width of the individual peaks reflects the distribution of the size of molecules for a given resin and its components. The distribution curve is also known as the molecular weight distribution (MWD) curve. Taken together the peaks reflect the MWD of a sample. The broader the MWD, the broader the peaks become and vice versa. The higher the average molecular weight, the further along the molecular weight axis the curve shifts and vice versa.

The Size Separation Mechanism

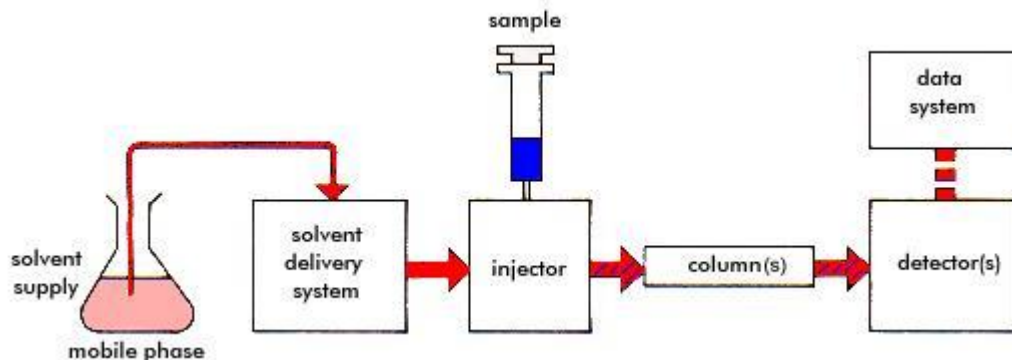


Molecules of various sizes elute from the column at different rates. The column retains low molecular weight material (small black dots) longer than the high molecular weight material (large black dots). The time it takes for a specific fraction to elute is called its "retention time".

3. GPC Systems

In designing instrumentation for GPC, a variety of requirements must be satisfied. Injectors are needed to introduce the polymer solution into the flowing system. Pumps deliver the sample and solvent through the columns and system. Detectors monitor and record the separation. Data acquisition accessories control the test automatically, record the results, and calculate the molecular weight averages. The gel permeation chromatograph contains a number of different components that work together to provide optimum system performance with minimum effort.

Schematic of a basic gel permeation chromatograph



This diagram illustrates how the sample is injected into the mobile phase and the path the sample takes to the detector.

A. Pump

Pumps the polymer in solution through the system.

Always be sure you have enough mobile phase for your run. Do not let the column run dry.

B. Injector

Introduces the polymer solution into the mobile phase.

250 μ l sample loop. Inject 100 to 200 μ l.

C. Column Set

Efficiently separates sample components from one another.

High efficiency columns give maximum separating capability and rapid analyses. Every column must provide reproducible information over extended periods for both analytical and fraction collecting purposes. We have 3 Styragel HR columns from Waters.

D. Detector

Monitors the separation and responds to components as they elute from the column.

In addition, the detectors must be sensitive and have a wide linear range in order to respond to both trace amounts and large quantities of material if necessary.

Since all compounds refract light, the differential refractometer (RI) is referred to as a "universal" detector. As a result it is the most widely used detector to monitor molecular weight distribution. The refractive index of polymers is constant above approximately 1000 MW. Therefore, the detector response is directly proportional to concentration. We have a Water 410 RI detector.

Beside information about molecular weight averages and distribution obtained with RI, the use of UV absorbance detectors may provide information about composition. We have a Waters 2996 PDI detector.

E. Automatic data processing equipment

Automatically calculates, records, and report numerical values for Mz, Mw, Mv, Mn, and MWD.

Data systems can also provide complete control of GPC systems so that large numbers of samples can be run unattended and raw data can be automatically processed. Today's GPC software offerings need to be able to provide special calculations for multi-detection processing, band broadening correction, special calibration routines and polymer branching determination, just to name a few.

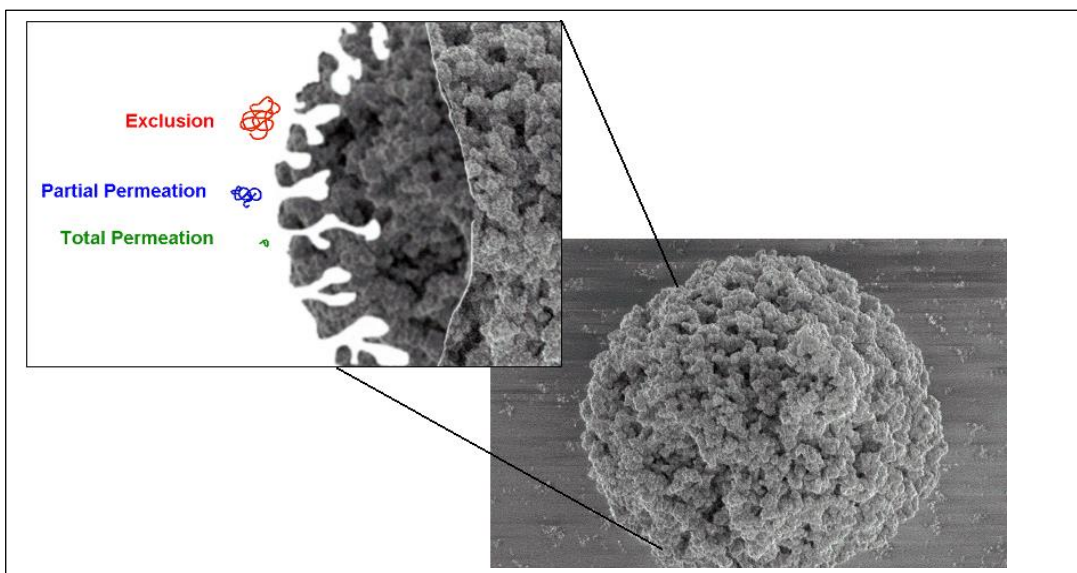
4. GPC/SEC Separations - Theory and System Considerations

Introduction to size separation:

Gel Permeation Chromatography, (GPC), also known as Size Exclusion Chromatography, (SEC) is really the easiest to understand of all the liquid chromatographic techniques. The separation is based strictly on the size of the sample in solution, and there should be no interaction with the column packing, (adsorption, partition, etc.), as you have with conventional HPLC. The mode of separation is not based on molecular weight, but on the size of the material being analyzed (usually a polymer) in solution. In other words, to do GPC correctly, the sample must be dissolved in a suitable solvent.

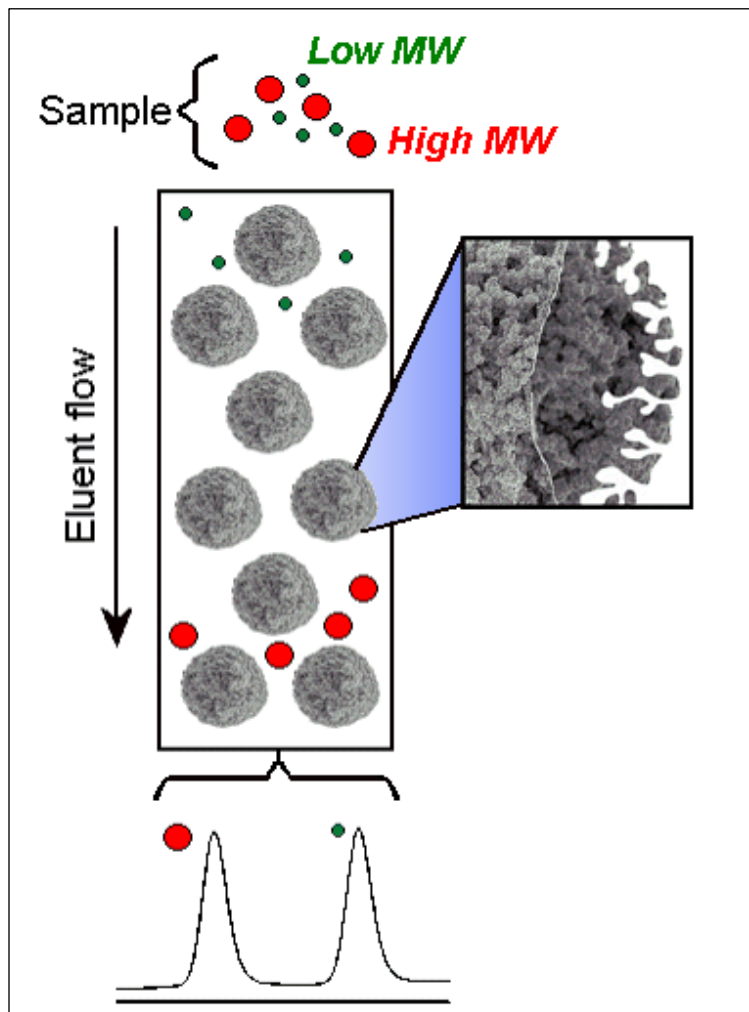
The concentration of the sample in solution depends on the molecular weight, but a concentration of 0.10% (w/v) for a polymer of molecular weight ~100,000, is typical. (See more in the Sample Prep. Section below).

Once the sample has been suitably dissolved, it is introduced via an injection mechanism onto a set of columns which act as a molecular filtration system. The columns are packed with a crosslinked gel, (styrene/divinylbenzene copolymer for organic applications), which contain surface pores. These pores can vary from small to quite large, and act as the molecular filter. The larger size molecules will not fit into the smaller pores. Conversely, the smaller molecules will fit into most of the pores, and be retained longer.



The larger molecules will elute first according to BOCOF's law (Big Ones Come Out First).

- Polymer coils in solution can permeate the pores on GPC packing materials
- Exclusion, partial permeation and total permeation are possible.



- Polymer is prepared as a dilute solution in the eluent and injected into the system
- The GPC column is packed with porous beads of controlled porosity and particle size
- Large molecules are not able to permeate all of the pores and have a shorter residence time in the column
- Small molecules permeate deep into the porous matrix and have a long residence time in the column
- Polymer molecules are separated according to molecular size, eluting largest first, smallest last
- As a result of the GPC separation mechanism, polymer molecules elute from the column in order of size in solution
- Largest elute first, smallest elute last

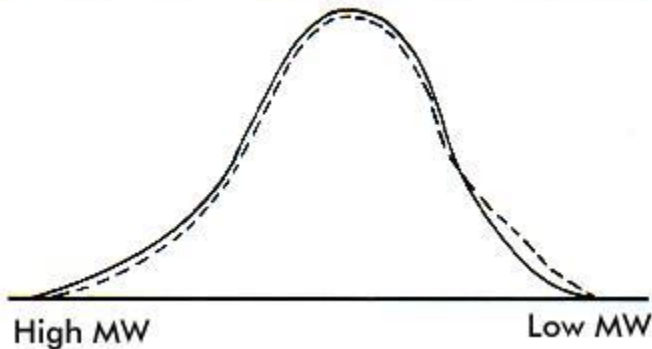
5. GPC Reports

Monomers, Oligomers, Polymers and Molecular Weight Distributions

Monomers have a single molecular weight, and are said to be monodisperse. Examples would be ethylene, styrene, vinyl chloride, etc. After monomers, we have dimers, trimers, tetramers, pentamers, etc., which are called oligomers. As we get to higher molecular weights, the group is called polymers. Polymers have a distribution of chain lengths, and, therefore, molecular weights. Depending on how the polymerization was carried out, this distribution can be narrow, or quite broad. As an example, a condensation, or step-growth polymer, such as a polyester, (polyethyleneterephthalate), will have a fairly narrow distribution of molecular weights. On the other hand, a free radical polymerization may produce a polymer with a very broad distribution of chain lengths and molecular weights, (such as for polyolefins). Controlling the kinetics of the polymerization is extremely important in obtaining a desired molecular weight distribution. That is why GPC is such an important technique to the polymer chemist.

Here we show an overlay of two molecular weight distributions of a polymer (in this case polystyrene):

Molecular Weight Distribution (MWD)



Molecular Weight Averages, M_n , M_w , M_z , M_{z+1} .

Polymers consist of repeat units (monomers) chemically bonded into long chains. Understanding the physical properties of a polymer (such as mechanical strength, solubility and brittleness) requires knowledge of the length of the polymer chains. Chain length is often expressed in terms of the molecular weight of the polymer chain, related to the relative molecular mass of the monomers and the number of monomers connected in the chain. However, all synthetic polymers are polydisperse in that they contain polymer chains of unequal length, and so the molecular weight is not a single value - the polymer exists as a distribution of chain lengths and molecular weights. The molecular weight of a polymer must therefore be described as some average molecular weight calculated from the molecular weights of all the chains in the sample. This overview describes the commonly used molecular weight averages that can be determined by gel permeation chromatography (GPC) and size exclusion chromatography (SEC), how they are defined, and the classical methods originally used to measure them.

Number average molecular weight: M_n

The number average molecular weight is the statistical average molecular weight of all the polymer chains in the sample, and is defined by:

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

where M_i is the molecular weight of a chain and N_i is the number of chains of that molecular weight. M_n can be predicted by polymerization mechanisms and is measured by methods that determine the number of molecules in a sample of a given weight; for example, colligative methods such as end-group assay. If M_n is quoted for a molecular weight distribution, there are equal numbers of molecules on either side of M_n in the distribution.

Weight average molecular weight: M_w

The weight average molecular weight is defined by:

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

Compared to M_n , M_w takes into account the molecular weight of a chain in determining contributions to the molecular weight average. The more massive the chain, the more the chain contributes to M_w . M_w is determined by methods that are sensitive to the molecular size rather than just their number, such as light scattering techniques. If M_w is quoted for a molecular weight distribution, there is an equal weight of molecules on either side of M_w in the distribution.

Higher average molecular weights: M_z , M_{z+1}

In general, a series of average molecular weights can be defined by the equation:

$$M = \frac{\sum N_i M_i^{n+1}}{\sum N_i M_i^n}$$

where: $n = 1$ gives $M = M_w$
 $n = 2$ gives $M = M_z$
 $n = 3$ gives $M = M_{z+1}$

The higher averages are increasingly more sensitive to high molecular weight polymers and accordingly are increasingly more difficult to measure with precision. They tend to be associated with methods that measure the motion of polymer molecules, such as diffusion or sedimentation techniques. Although the z-averages are not commonly quoted for polymers, several important methods for measuring the dimensions of chains that yield z-average molecular weights.

For all synthetic polydisperse polymers:

$$M_n < M_w < M_z < M_{z+1}$$

The polydispersity index is used as a measure of the broadness of a molecular weight distribution of a polymer, and is defined by:

$$\text{Polydispersity index} = \frac{M_w}{M_n}$$

The larger the polydispersity index, the broader the molecular weight. A monodisperse polymer where all the chain lengths are equal (such as a protein) has an $M_w/M_n = 1$. The best controlled synthetic polymers (narrow polymers used for calibrations) have M_w/M_n of 1.02 to 1.10. Step polymerization reactions typically yield values of M_w/M_n of around 2.0, whereas chain reactions yield M_w/M_n values between 1.5 and 20.

Molecular weight averages and molar mass distributions

Simple transfer of the sample elution volume into the peak apex molecular weight M_p is not sufficient because it characterizes the sample only in a single point. For better characterization the eluted peak is divided into several equidistant volume slices and the molecular weight averages are calculated as shown in the equations on the right, where $h(M)$ is the slice height at a molecular weight M . The most important averages are M_n and M_w . M_n provides information on the flexibility and M_w on the strength of the material. The molecular weight averages describe the polymer at different points of the peak. This can also be achieved using traditional techniques such as membrane osmometry or light scattering. GPCSEC, however, is the only technique which in addition yields the molecular weight distribution. This is a plot of the statistical frequency of molecular weights versus the log of the molecular weight. The molecular weight or molar mass distribution is most important to characterize polymers. The molecular weight averages describe only average properties of the sample. Figure 3 shows the molar mass distributions of three polymers with identical molecular weight averages. The completely different molar mass distributions indicate clearly that they have different properties.

$$\text{Number average molecular weight: } M_n = \frac{\sum h(M) \cdot M}{\sum h(M)} = \frac{\sum w(M)}{\sum w(M)/M}$$

$$\text{Weight average molecular weight: } M_w = \frac{\sum h(M) \cdot M^2}{\sum h(M) \cdot M} = \frac{\sum w(M) \cdot M}{\sum w(M)}$$

$$\text{z-average molecular weight: } M_z = \frac{\sum h(M) \cdot M^3}{\sum h(M) \cdot M^2} = \frac{\sum w(M) \cdot M^2}{\sum w(M) \cdot M}$$

$$\text{Viscosity average molecular weight: } M_v = \left(\frac{\sum w(M) \cdot M^a}{\sum w(M)} \right)^{1/a}$$

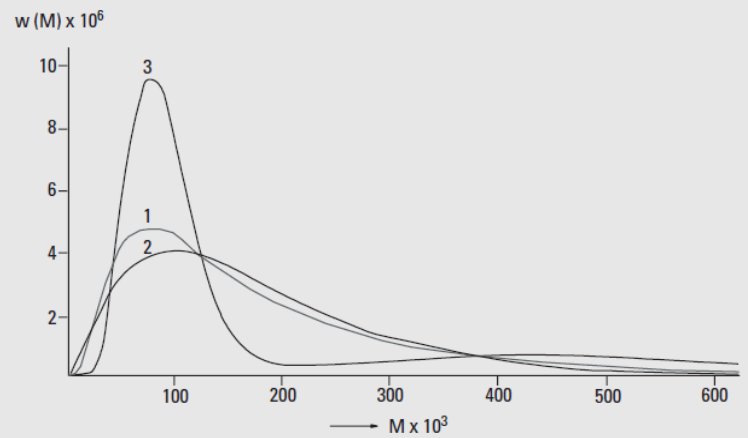


Figure 3
Molar mass distributions of three polymers with the same molecular weight averages

There are other techniques to obtain these molecular weight averages:

- Number average, M_n , may be obtained by membrane osmometry, or end group analysis, (titration, NMR, etc.)
 - Weight Average, M_w , may be obtained by light scattering
 - Z Average, M_z , and Z + 1 Average, M_{z+1} , may be obtained by ultracentrifugation
- Once we have calibrated our GPC system, we can obtain all of these averages with a single injection.

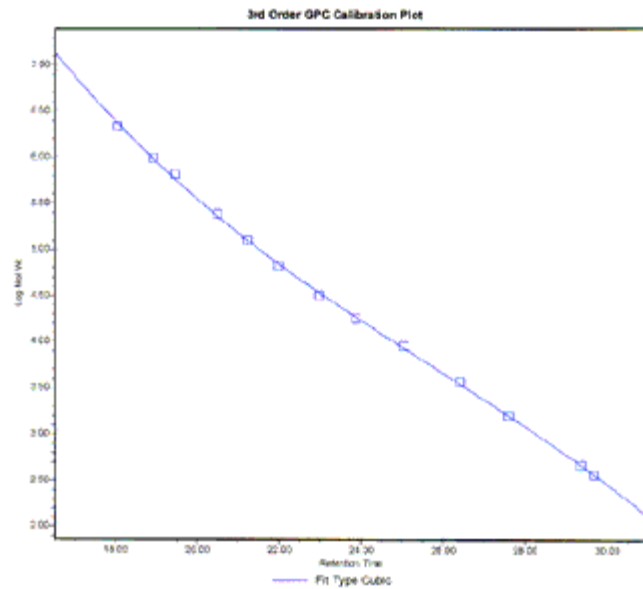
6. Calibration of the GPC System

In order to assign a molecular weight to each retention time slice for the eluted polymer, we must calibrate our system, or more specifically, the column set. There are several ways to do this, but the easiest is to use a relative calibration based on a set of well-characterized polymer standards with as narrow a molecular weight distribution as possible. Ideally, we would like to use a set of standards that are monodisperse, i.e., a single molecular weight, with the weight and number average ratio (dispersity) being equal to one, ($M_w/M_n = 1$).

The closest we can come to achieving this is to use polymer standards that are polymerized specifically for this purpose, such as the anionically polymerized polystyrene narrow standards. Standards cover a very broad molecular weight range, from monomer to molecular weights > 10,000,000, with a dispersity of < 1.10. For a calibration standard to be really considered narrow, and acceptable for use in GPC calibration, the dispersity should indeed be < 1.10.

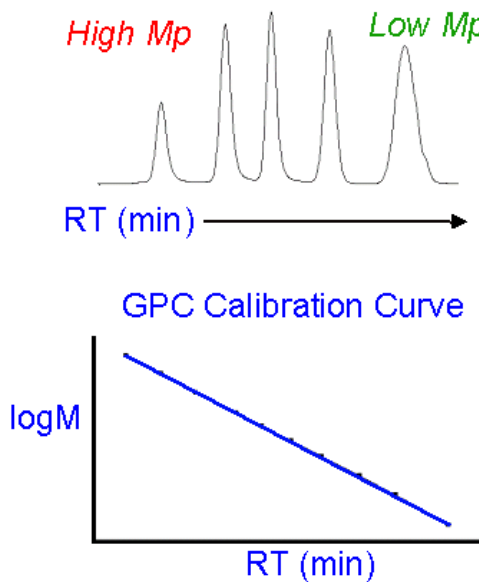
Relative, Narrow Standard Calibration

We call the conventional narrow standard calibration technique a relative calibration because the molecular weight averages obtained are relative to the calibrant. For example, if one were running polyethylene as a sample, and calibrated the column set with polystyrene narrow standards, the molecular weights obtained after integration would be based on polystyrene, and incorrect for polyethylene. This is fine for many people, however, who are simply comparing molecular weights obtained for an unknown against a set of "acceptable" values. Whether these molecular weight values are really "absolute" for their polymer of interest is unimportant; just as long as these values obtained are in the acceptable range.



There are a few other narrow standards available for organic GPC, such as poly(methylmethacrylates), polyisoprenes, polybutadienes, poly(THF), but certainly polystyrene is the major narrow standard used for organic GPC analysis. In the case of aqueous GPC, poly(ethylene oxides) are the most widely used, along with poly(ethylene glycols) for low molecular weight, and the pullulans, which are polysaccharides based on triose structures. After running the series of narrow standards, a polynomial fit is then performed, (usually third or fifth order), and the resulting log M vs. retention time (or volume) calibration curve is plotted.

Chromatogram of narrow standards



- Chromatograph a series of well characterised, narrow polydispersity polymer standards
- Plot peak retention time (RT) versus peak log molecular weight (logM)
- Fit the data using a mathematical function
- The calibration curve will be characteristic of the GPC column set used

7. GPC Sample Preparation

The most important criteria in preparing to do a GPC analysis is finding a suitable solvent to dissolve the polymer. This sounds trivial enough, but remember that GPC is a separation technique based on the size of the polymer in solution. Polymer chains will open up to a certain relaxed conformation in solution, and the solvent chosen will determine what this size will be. Many polymers are soluble at room temperature in various solvents, but in some cases, (especially for highly crystalline polymers), high temperature is required for dissolution. Another important aspect for GPC sample preparation is the concentration chosen. If the mass loading of the sample onto the column set is too high, there may be concentration or viscosity effects, which will give rise to incorrect elution volumes. Another consideration is whether or not to filter the polymer solution. We will discuss some of these sample preparation considerations.

One should always filter the eluent under vacuum before use in the chromatographic system. With the organic solvents, a fluorocarbon filter is generally used. The filter pore membrane size is generally 0.45m (micron).

A. Concentration

Once we have chosen the proper solvent for the analysis, the next step is to prepare the narrow standard and sample solutions. We need to be careful to use enough concentration to be able to get an acceptable signal-to-noise, but at no risk of overloading the column and risking concentration effects. The table below is a general "rule of thumb" to be used as a guide as to what concentration should be prepared. These concentrations are in percent, where 1.0 mg/ml is 0.10%. No correction is made for temperature, so everything is assumed to be prepared at room temperature. These concentrations shown are to be used assuming a maximum of 100ul injection volume per column.

Molecular Weight Range	Concentration Range (weight per volume) w/v
MW > 1,000,000	0.007- 0.02%
500K - 1,000,000	0.02 - 0.07%
100K - 500K	0.07 - 0.10%
50K - 100K	0.10 - 0.13%
10K - 50K	0.13 - 0.16%
<10K	0.16 - 0.20%

B. Preparing the Sample

Now that we have successfully dissolved the standards and samples in our chosen solvent, and have installed our GPC columns, we are ready to start making injections. The next choice we have to make is whether or not we should filter the sample solution. In nearly all cases, we should filter the sample solution prior to injection.

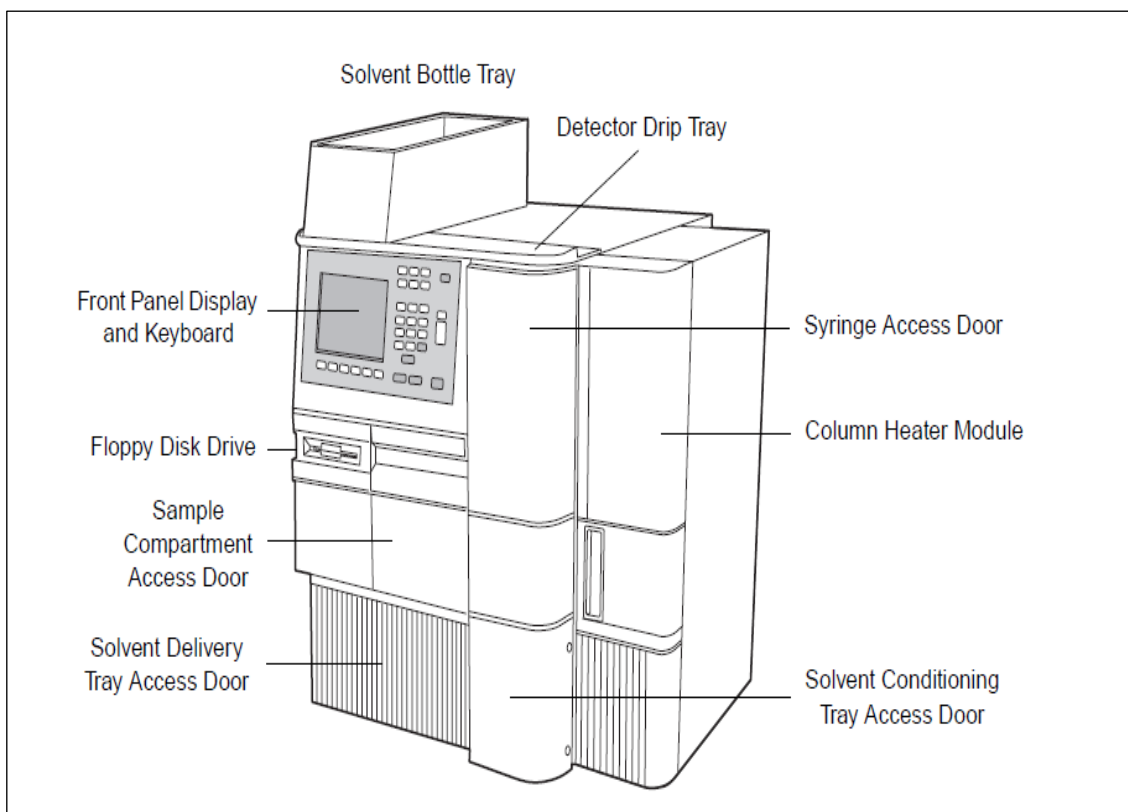
Generally, as in the case of the solvent filtration discussed previously, we would choose a 0.45 m membrane fluorocarbon filter. In some cases, where there is very fine particulate material (such as carbon black, titanium dioxide, silica, or other fillers), a 0.45 m filter may be used.

Obviously, when we start to use very fine filter sizes, polymer shear may become a concern. Filtering a high molecular weight polymer through a 0.20m filter would certainly cause some shear degradation. One may have to choose not to filter the sample at all, and hope there is no pressure increase due to plugging of the system in-line filter or column frit.

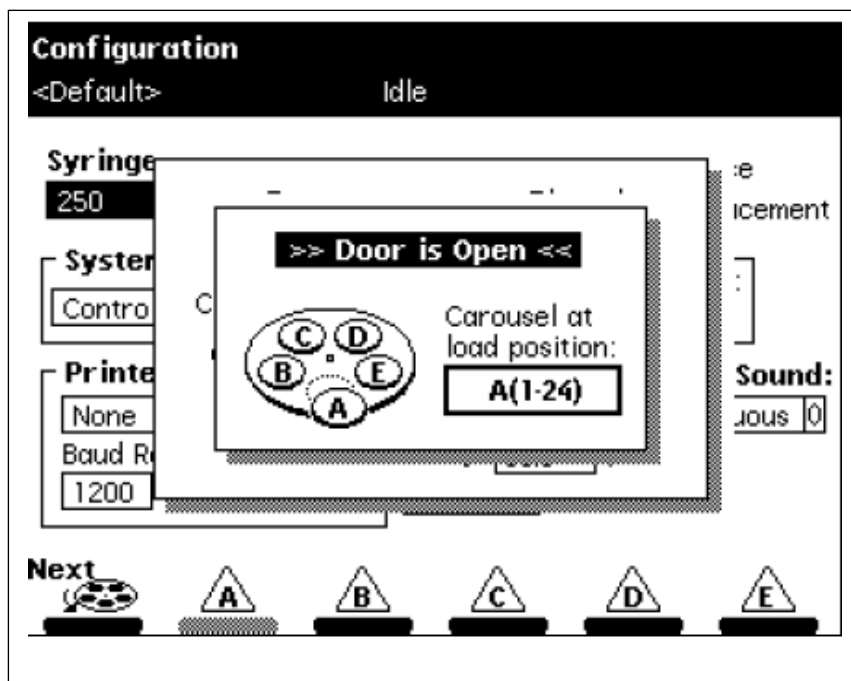
Now we can start making injections of the standards and samples. As mentioned previously, we will inject a maximum of 100ul per column, at the concentrations shown in the table. Our run time will be approximately 15 minutes per column at a flow rate of 1.0 mL/min so the analysis time for a three column set would be ~45 min.

Once the sample set has been run, it is time for the data handling system to process the results according to the integration method we designated and furnish a completed report. This can be done automatically in a "Run and Report" mode in Empower Software, or we may choose to go in to each raw data file and manually integrate each sample.

8. Alliance 2695 Waters HPLC Instrument



- A. Sample compartment
 - a. Removing the Carousel.
 - b. Open front compartment door. The Door is Open box appears on screen.



- c. Select the desired carousel tray button by pressing the A, B, C, D or E triangle.
- d. Pull out carousel and add sample vials.
- e. Place carousel back in compartment and close door.

9. Columns

Waters Styragel HR GPC columns, 5 μ m, 7.8mm x 300mm:

HR1 = 100 to 5K

HR3= 500 to 30K

HR 4= 5K to 600K