Polymer analysis by GPC-SEC

Technical Note

Introduction

Gel Permeation Chromatography (GPC), also referred to as Size Exclusion Chromatography (SEC) is a mode of liquid chromatography in which the components of a mixture are separated on the basis of size. In GPC-SEC large molecules elute from the column first, followed by smaller molecules. It is an important tool for the analysis of polymers. The essential results are molecular weight data and molecular weight distribution curves which are needed to characterize a polymer with regard to differences in properties. GPC-SEC is mainly used for samples with a molecular weight above 2000 although it is also in use for oligomer separations. There is no upper limit in the molecular weight, even polymer analyses with molecular weights of several millions are possible. Demands on the instrumentation are very stringent due to a special calibration procedure using a linear elution volume on the x-axis versus a logarithmic molecular weight on the y-axis.

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Mechanism

The column packing for GPC-SEC is a rigid or semi-rigid totally porous material with pores of known size. Figure 1 illustrates the mechanism. The pores are conical in shape, which is not necessarily the case in reality. The example shows a mixture which contains three components A, B and C with A being the largest and C being the smallest. As the components are carried through the column by the mobile phase, component A cannot diffuse into the pores, (that is, it is excluded), component B may diffuse approximately halfway into the pores, (that is, it partially permeates) and component C may diffuse all the way into the pores (that is, it permeates totally). Thus the order of elution from the column would be A, then B, and then C.

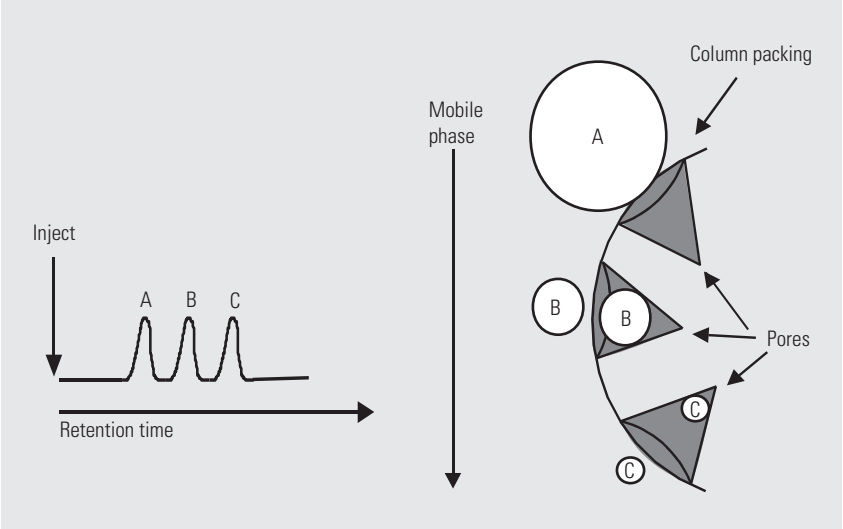

Figure 1
GPC-SEC separation mechanism

Molecular weight correlation: calibration

The separation mechanism in GPC-SEC is based on the size of the molecule when solvated by the mobile phase. A correlation can be made between size and molecular weight. Figure 2 shows that a plot of logM against retention volume is linear for components that selectively permeate the column packing pores. From a calibration plot and the retention volume of the sample, its molecular weight or molecular weight range can be determined.

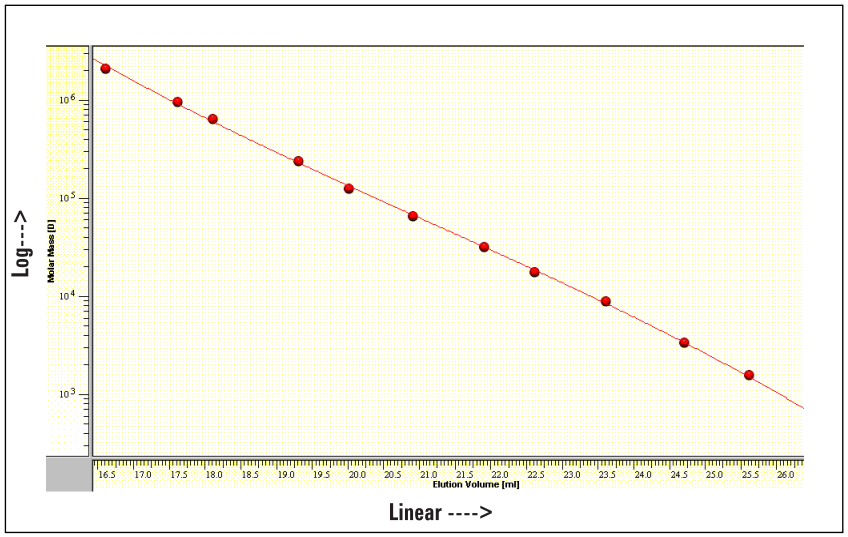

Figure 2
Typical GPC-SEC calibration plot

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. GPC-SEC, 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.

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

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

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

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

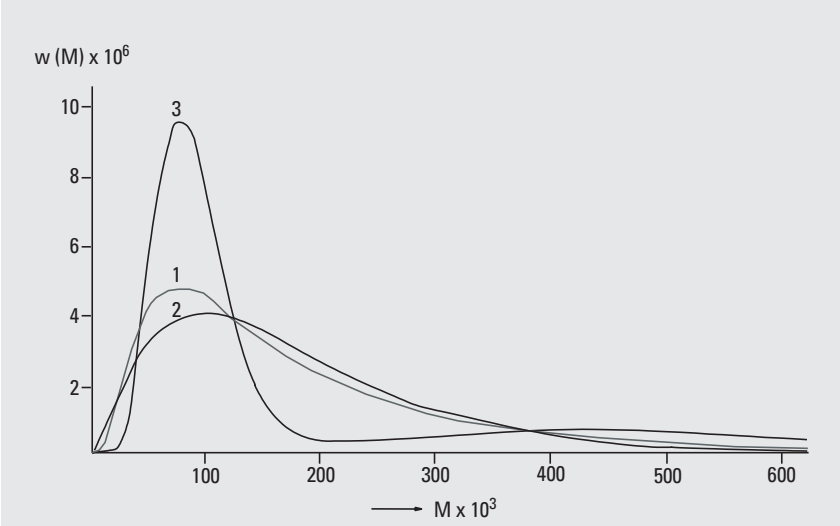

Figure 3
Molar mass distributions of three polymers with the same molecular weight averages

Mobile phase selection

In theory, the mobile phase serves only to dissolve the sample and carry it through the column. In other modes of HPLC, such as partition, adsorption and ion-exchange, there is interaction between the mobile phase and the stationary phase on the column packing and retention can be varied by changing the strength of the mobile phase. In GPC-SEC a change in mobile phase may cause a relatively small change in retention due to a change in hydrodynamic volume of the sample in different mobile phases. Also, a change in mobile phase may cause a change in pore size of the gel packing due to swelling or shrinking of the gel. These changes in retention are very small compared to the changes seen in the other HPLC modes. In GPC-SEC the mobile phase serves only to dissolve the sample and carry it through the column and a change in solvent produces a relatively small change in retention. Therefore, gradient elution is not used.

The mobile phases can be roughly devided into organic and aqueous mobile phases. Tetrahydrofuran(THF) is the most frequently used organic solvent. It is used for a wide range of polymers as polystyrene, poly(methyl metacrylate), epoxy resins, polycarbonate, polyvinylchloride, and polystyreneacrylonitrile. Other solvents include toluene, dimethylacetamide and dimethylformamide. For more information on mobile phases and columns recommended for a wide selection of polymer, see reference 1.

Column packings

Two general types of column packings are available: polymeric gels and silica gels. There are advantages and limitations to both types of packings. Polymeric gels are widely used. Adsorption effects are negligible, however, there are restrictions on solvents that can be used with these gels. Also, the gels can be damaged by pressure "shocks" since they are compressible. The silica packings are more stable physically and are compatible with a wide range of mobile phases. However, adsorption can be a problem with the silica packings unless the surface is deactivated. Highly-crosslinked polystyrene/divinylbenzene particles as packed in the Agilent PLgel columns are among the most widely used columns for polymer separations with organic mobile phases. They are available with different particle and pore sizes to cover a wide range of polymer molecular weight distributions (figure 4). For the analysis of broad distributed polymers one column alone is not sufficient. Such wide ranges usually require sets of several columns, typically between two to three (up to six). For more information on mobile phases and columns recommended for a wide selection of polymers, see reference 1.

An alternative to polystyrene/divinylbenzene based stationary phases are the ZORBAX PSM phases, which are available as small (5 µm) porous microspheres (PSM) in a deactivated and an untreated version. The deactivated version has been silanized for use with non-polar to relatively polar polymers in non-aqueous or partially aqueous solvents. The untreated version is for use with both non-aqueous and aqueous

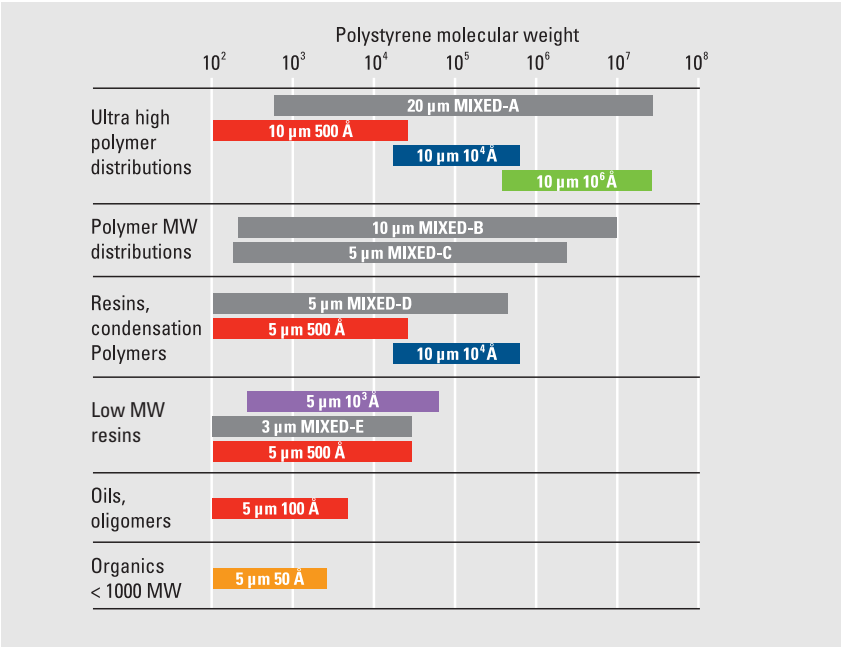

Figure 4
Molecular weight application range of PLgel columns

mobile phases. Dedicated to analyses with aqueous mobile phases are the Agilent PL aquagel-OH columns with their extremely hydrophilic polyhydroxyl surface. They can handle most neutral hydrophilic polymers, and the capability extends to the analysis of high molecular weight polymers (figure 5) including polyacrylamides and polyethylene oxides.

Instrument requirements

Due to the special calibration procedure using a linear elution volume (retention time) on the x-axis versus a logarithmic molecular weight on the y-axis the requirements on hardware and software are very demanding. Accuracy and precision of molecular weight data depends on several hard- and software parameters as listed in table 1.

One of the most important parameters is flow precision. Table 2 shows the strong influence of flow deviations on the weight average molecular weight M_w measured for a polystyrene sample.
The system was calibrated at a flow rate of 1.0 ml/min. When analyzed at exactly this flow rate the M_w value is 35400. Table 2 shows that for a flow deviation of only +0.60 % or +1.30 % errors of 11.0 and even 23.6 % occur.

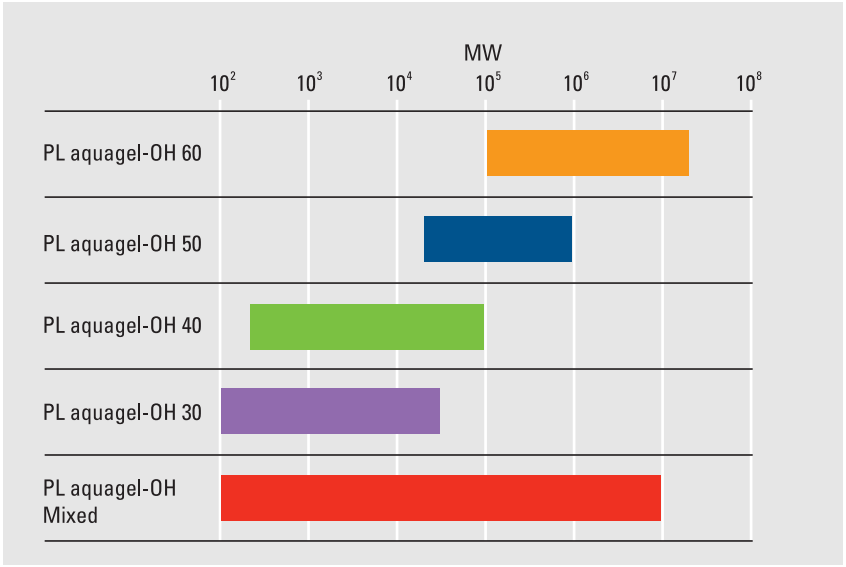

Figure 5
Molecular weight application range of PL aquagel-OH columns

Hardware Parameters	Software Parameters
• Column stability • Precise pump flow with retention time precision < 0.1 % • Column temperature precision ± 0.15 °C • Lowest short-term and long-term noise • Autosampler with low maintenance	• Precision of calculation procedures • Precision of baseline setting • Precision of setting the calculation start and end marks • Number of data points user selectable • Various calibration routines • Automated and interactive data analysis and reporting • Possibility to use an internal standard correction for flow rate changes

Table 1
Hardware and software parameters influencing accuracy and precision of molecular weight data

Flow [ml/min]	Flow deviation [%]	M_w	M_w deviation [%]
1.013	+1.30	43400	+23.6
1.006	+0.60	39300	+11.0
1.0	0	35400	0
0.992	-0.80	31100	-12.2
0.985	-1.50	27700	-21.80

Table 2
Influence of flow variations on molecular weight

Column temperature stability between calibration and sample run is also important. A 4 °C change, as it can easily occur if the column compartment is not thermostated, will create an error of 2.6 %.

On the software side it is important that the software is correctly installed and calculates correctly. State-of-the art GPC-SEC software therefore offers installation verification and system verification routines. The installation verification routine should be performed after installation and later on periodically to prove that all parts are correctly installed. System verification is used to prove that the software is calculating properly. A data file and a calibration file–provided as a protected part of the program–will be processed and a report will be generated as a printout. The GPC raw data from the known sample is processed in exactly the same way as data acquired by the Agilent ChemStation. This ensures that not only the final calculations are verified but also the complete data processing path. The results are then compared to the theoretical results and the system verification test is only passed if results differ less than a specified percentage. Hardware and software parameter effects on accuracy and precision of molecular weight data are discussed further in references 2 and 3.

Refractive index detection is most frequently used for polymer characterization by GPC-SEC. Some polymers, such as polyethyleneoxides, dextrans, celluloses, do not absorb in the UV-visible range.

On the other hand there are several polymers, that can be analyzed with UV-visible detection provided the eluent is transparent and the correct detection wavelength is selected. Examples are aromatic groups containing polymers as polystyrenes or poly(styreneacrylonitrile)s but also polymers without aromatic groups such as poly(methyl methacrylate)s, polybutadienes, polycarbonates, polyamides and polyacrylic acids. Figure 6 shows an overlay of a poly(methyl methacrylate) (PMMA) analysis obtained with refractive index and UV detection. One advantage of dual detection is that the operator receives more information about the sample. The PMMA chromatograms are very similar in the polymer region but show distinct differences in the oligomer region due to the better sensitivity of the UV detector.

If the UV detector is a diode array detector spectra can be acquired during the analysis and used for peak identification and peak purity control. For an example refer to reference 5. A further advantage of UV-visible detection is lower baseline noise and drift. This should have an influence on the accuracy and the precision of the molecular weight data.

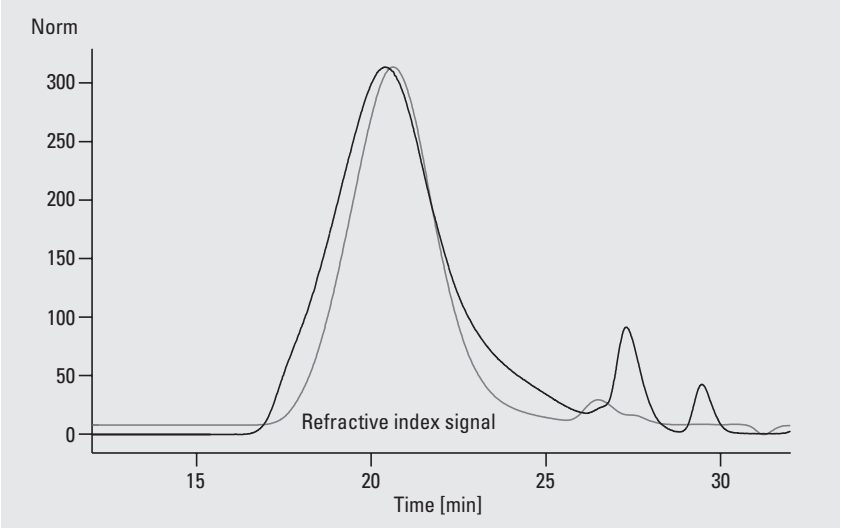

Figure 6
Overlay of poly(methyl metacrylate) chromatograms obtained with UV and refractive index detection

To study this we analyzed a technical polystyrene sample with UV-DAD-and refractive index detection in series. Table 3 shows the average M_n and M_w values and the respective relative standard deviations calculated from 10 automatic analyses.

State-of-the-art refractive detection has significantly improved in terms of baseline noise, wander, drift and automation capabilities. Therefore, the data in table 3 is very similar for the two detectors with some difference in the precision data. The precision data for UV-visible detection is typically better than the refractive index detection data by a factor of approximately two.

	Average value		Precision	
	M_n	M_w	M_n	M_w
Reference value	86000 (GPC)	246000 (light scattering)	-	-
UV-DAD	90700	265000	0.69	0.33
RID	91530	265000	1.24	0.36

Table 3
Comparison of accuracy and precision obtained with UV-DAD and refractive index detection

Conclusion

GPC-SEC is the most widely used technique for the analysis of polymers. It can be used for samples soluble in organic and aqueous eluents and molecular weights from approximately 100 to several million Dalton. In contrast to traditional techniques it yields all molecular weight averages and the molecular weight distribution. To obtain accurate and reliable results the demands on hardware and software are more stringent than for other HPLC modes.

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Printed 10/2000
Publication Number 5988-0110EN

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