Polymer Analysis by Gel Permeation Chromatography — A Historical Perspective

The development of polymer has had a profound effect on the modern world. These versatile materials are used in an extensive array of products ranging from drinks bottles and compact discs to shampoo additives and prosthetic hip joints. The first truly synthetic polymer was Bakelite, developed by Beakeland in 1907 from carbolic acid and formaldehyde. In the 1950s, advances in polymer science led to the development of a wide range of what are now bulk synthetic polymers such as polystyrene and poly(vinyl chloride), still very widely used today. Engineering polymers like polyethylene, polypropylene and polyurethane followed in the 1950–60s, and have become very high tonnage commodities. Recent developments have been in the area of speciality high technology materials that display high thermal and chemical stabilities, such as polyaryletherketones, polyimides and polytetrafluoroethylene. Literally thousands of different classes of polymer have been developed using a range of different chemistries, making plastics the most versatile of materials.

The increase in the popularity and use of plastics has necessitated the need to develop methods to fully characterize these materials. There are many parameters which affect the performance of polymers, including melt viscosity, toughness and glass transition temperature. One property that strongly influences many other parameters is the molecular weight distribution, that is the distribution of chain lengths in the sample. This article discusses the development of gel permeation chromatography (GPC) or size exclusion chromatography (SEC) as a technique for analysing the molecular weight distributions of polymers. (In the context of this article, the term GPC/SEC shall be used to describe the technique by which polymers are separated by molecular size in solution via entropic partitioning in a porous matrix.) This paper will discuss the development of columns and hardware for high-resolution characterization of polymers.

Column Technologies for GPC

Chromatographic techniques for separating polymeric materials on the basis of size in solution were first developed in the late 1950s and early 1960s using polydextran and polyacrylamide

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"soft gel" materials. The technique was named "gel filtration chromatography" (GFC) and was used to separate water soluble polymers. These soft gels have a "microporous" structure, in which a low degree of crosslinking (typically under 10%) yields a non-rigid material with the pore structure introduced by swelling in solvent. Soft gels display low mechanical stability that has restricted their use to applications involving low flow-rates and long analysis times. They are also relatively intolerant to extremes of pH and organic solvents. These materials are still employed for applications involving the analysis of water soluble polymers such as proteins and polyacrylamide. Agarose and polystyrene/divinyl benzene soft gels are readily available, however, the limitations inherent in these materials has led to the development of highly crosslinked macroporous materials.

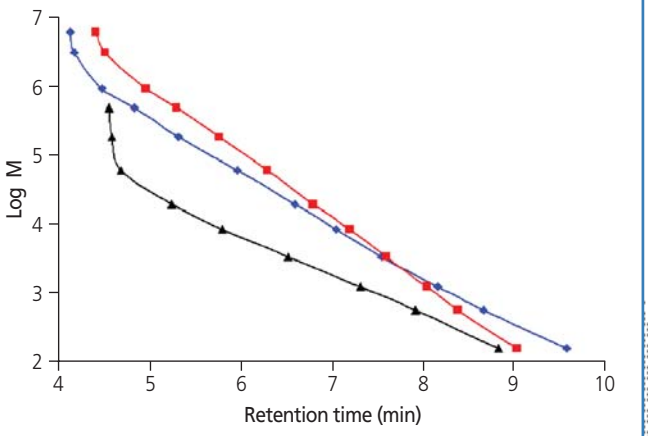
Macroporous semi-rigid particles for GPC were first developed by Moore. Synthesized by suspension polymerization, these materials have much higher crosslinking than the microporous gels, giving a rigid structure with limited swell. The pore structure is introduced by the use of porogens during synthesis and is well defined compared with the soft gels; as a result, semi-rigid particles are mechanically strong and can be used in chromatography at higher flow-rates. The pore structure can also be tailored during synthesis, generating materials with a range of pore sizes. Figure 1 compares and contrasts micro and macroporous materials.

As a result of the controlled pore sizes and mechanical stability of macroporous materials, these are far more widely used for size separations than microporous gels. They are available in a wide variety of chemistries from (relatively) polar to non-polar. The most popular are based upon co-polymers of styrene and divinyl benzene. Early suspension polymerizations

Figure 1. Comparison of micro and macroporous materials for gel permeation/size-exclusion separations.

	Macroporous	Microporous
		
Crosslink density	High > 20%	Low 2–12%
Swell	Low	High
Pore size	Independent of eluent	Determined by eluent
Mechanical strength	Good	Poor
Operating conditions	High pressure, low flow	Low pressure, low flow
Examples	PS/DVB	Polydextrans, polyacrylamides

Figure 2. Overlaid calibration curves of typical individual, mixed bed and multipore GPC columns (Polymer Laboratories' PLgel 5 µm 500Å, PLgel 5 µm MIXED-C and PolyPore columns).



produced macroporous gels with a narrow pore size distribution and a fairly narrow particle size distribution. A range of manufacturers commercially produce these "individual pore size" gels.

GPC columns packed with individual pore size materials are quite versatile in application, being robust enough for use in a wide variety of organic solvents. As a result, these gels are popular and are still in use today. The limitation of individual pore size materials is that they only give resolution over a short range of molecular weight. In order to increase the resolving range of the separation, several columns of different pore size must be combined together in series, for example, a column set comprising a PLgel 5 µm 10^6 Å, 10^4 Å and 500 Å resolves from over 10 000 000 g mol^{-1} down to 500 g mol^{-1} (polystyrene equivalent in THF).

Another characteristic of individual pore size materials is the artefacts that can occur when combining columns together because of the overlap of the resolving range of the columns. Mismatches in pore volume in these regions can manifest as shoulders on chromatograms known as "dislocations". In the early 1980s, a new column type was introduced to address this limitation, the "linear" or "mixed bed" column. In these columns, a homogenized mixture of individual pore size gels is introduced to cover the desired resolving range, with the proportion of each gel carefully selected to ensure that the resulting calibration curve is linear, and that no dislocations occur.

Since the development of mixed bed columns, improvements have been made in manufacturing processes and product quality. Recently there has been a new development in organic GPC with the introduction of "multipore" gels. These materials are composed of a single gel type (as with individual pore size materials) but each polymeric particle contains a wide range of pore sizes. Consequently, multipore columns packed with a single type of material have very wide resolving ranges, removing the necessity of blending materials (as in mixed bed or linear columns) and eliminating the possibility of dislocations. A further advantage to the methods used to manufacture these gels is that the pore volume is very high, leading to an increase in resolution when compared with traditional GPC materials. Such multipore materials are the

Figure 3. The PL-GPC 50.

cutting edge in GPC column technology.

Aqueous GPC columns have been developed in parallel with the organic products. Of the three column types discussed above, aqueous columns based on "individual pore size" and "mixed bed" gels are available, but there are currently no available multipore materials. For GPC columns to work successfully in water, the hydrophilicity of the packing material must be increased significantly. Polymeric-based gels are typically highly non-polar and so some modification of the gel is required, either in terms of secondary treatment of organic gel or alteration of the synthesis stage to use different monomers. For many aqueous separations, silica based materials are still popular.

To illustrate the range of commercial columns available, Table 1 shows examples of column ranges from various manufacturers (the list should in no way be considered exhaustive). Figure 2 shows an overlay of calibration curves for typical individual pore size, mixed bed and multipore columns (Polymer Laboratories' PLgel 5 µm 500 Å, PLgel 5 µm MIXED-C and PolyPore columns).

Hardware Technologies for GPC

The first systems used for GPC were home-made instruments, as with other high performance liquid chromatography (HPLC) systems of the time. As development progressed, manufacturers introduced "modular" GPC equipment, comprising of a separate pump, injection and detector modules. Other ancillary devices such as autosamplers and column ovens, for example, can also be added to these systems. The advantage of modular systems is that components may be mixed and matched between manufacturers, however, the disadvantages are that there is no communication between instruments and the user must learn to control the interface of each device.

In parallel to the development of other liquid chromatographic techniques, the emergence of integrated

systems specifically designed for GPC has been the trend over the last ten years. These instruments contain all the components required for the analysis (pump, injection system, column compartment, detectors and ancillary devices) within one unit. The benefit of such devices is that the positioning of the components can be optimized to produce the best results, various safety features can be introduced and custom computer software can be developed to control every part of the system. Such instruments are, therefore, easier to use (and require less training) than modular systems and contain considerably more safety features. They can also be tailored to suit particular analyses such as high temperature work much more easily than modular systems. An example of an integrated system expressly designed for GPC is the PL-GPC 50 shown in Figure 3. This instrument can be operated from ambient to 50 °C and is designed to accommodate a range of detector options.

Table 1. Examples of column ranges from various manufacturers (the list should in no way be considered exhaustive).

Column type	Column range	Organic or Aqueous	Manufacturer
MultiPore	PlusPore	Organic	Polymer Laboratories
	TSK-GEL HXL	Organic	Tosoh Bioscience
	Shodex K-series	Organic	Showa Denko
Mixed / Individual	PLgel	Organic	Polymer Laboratories
	PL aquagel-OH	Aqueous	
	TSK-GEL HXL	Organic	Tosoh Bioscience
	TSK-GEL PW	Aqueous	
	Shodex K-series	Organic	Showa Denko
	Shodex OH pak	Aqueous	
	Styragel	Organic	Waters Corp.
	Ultrastyragel	Aqueous	

Table 2. Examples of commercially available integrated systems, light-scattering and viscometry detectors and software developed specifically for gel permeation chromatography (the list should in no way be considered exhaustive).

Product type	Name	Application	Manufacturer
Instruments	PL-GPC 50, 120, 220	Integrated GPC systems	Polymer Laboratories
	PL-BV 400, PL-ELS 1000	GPC detectors	
	TDA	Integrated GPC system	Viscotek
	LALS, Viscometers	GPC detectors	
	Alliance	Integrated GPC systems	Waters Corp.
	DAWN Series, ViscoStar	GPC detectors	Wyatt
Software	Cirrus	GPC and Multidetector	Polymer Laboratories
	OmniSEC	GPC and Multidetector	Viscotek
	Millennium	GPC and Multidetector	Waters Corp.

A GPC experiment employing only a single concentration detector can be termed conventional GPC. In this analysis, molecular weight averages are calculated relative to a series of standard polymers of known molecular weight. The disadvantage of this method is that the GPC column separates on the basis of size not molecular weight, which can lead to discrepancies if the standards used for calibration are a different chemistry (and therefore size in solution) to the sample. Of increasing interest over the last ten years has been so called "multi detector" GPC, employing one or more detectors that respond to molecular properties other than concentration. Light scattering detectors and viscometers are the most common detectors used in these applications, with the use of both devices (along with the required concentration detector) termed "triple detection".

Static light-scattering detectors respond directly to the molecular weight of the sample and so do not rely on a column calibration, and can also give information regarding the size of the polymer molecules in solution. The development of light-scattering theory is well established, yet the first commercial instrument was only available from the 1960s. Since that time, many instruments have been developed specifically for coupling to GPC systems, covering the range of designs and prices. As a consequence, light scattering has grown from a niche area of academic interest to a very popular technique.

Viscometry detectors can be employed to improve the accuracy of GPC molecular weight calculations by removing the dependence of the calculated molecular weights on the chemistry of the standards and sample. This is achieved using the Mark-Houwink relationship and the Universal Calibration principle. Viscometry also gives the intrinsic viscosity of the sample under investigation, a property that can be related to the molecular density of the polymer molecules. Flow-through (i.e., compatible with liquid chromatography) viscometry detectors were first developed in the 1960s and although many types exist, those which employ liquid flow through capillaries are by far the most common. The advantage of viscometry is that the technique can greatly improve the accuracy of a GPC analysis compared with a conventional experiment and the instruments tend to be robust and reliable. With new and more cost effective designs becoming available, viscometry is becoming increasingly common in the GPC laboratory.

The growing popularity of GPC and the development of integrated instrumentation has led to increased software requirements. Data collection in early GPC systems was through chart recorders, with data processing either by hand or through custom programming. Of course these methods were clearly not suitable for widespread use and as GPC grew in popularity, data collection hardware and data handling software have been developed. Many companies with an interest in GPC have released data collection and handling software that is modern, powerful and versatile, and software now represents a

major component of the modern GPC system. The majority of these GPC software packages are intuitive to use and Windows® based, with some companies offering to fill compliance and regulatory requirements where necessary.

As an example of the hardware available, Table 2 shows a summary of the integrated systems, detectors and software available specifically for gel permeation chromatography.

Conclusions

Initially developed for the separation of water soluble polymers by size in aqueous solution, the technique of gel permeation chromatography has been greatly developed to allow the analysis of a huge variety of synthetic and natural polymers. Early microporous soft gels have been replaced in the majority of applications with more robust semi-rigid macroporous polymeric materials that allow analysis in a wide range of solvents. The development of mixed bed or linear columns and now multipore high pore volume material has greatly increased the versatility of GPC. Progress in the design and technology of GPC instruments has occurred to match this growth in interest. Integrated instruments specifically designed for GPC have superseded the early modular systems, and several companies now produce light scattering and viscometry detectors, instruments once only available in dedicated research laboratories. Specialized GPC software is available that greatly enhances the ease-of-use of GPC.

Since its inception, GPC has grown steadily in popularity to become a mainstay of the analytical laboratory. The growing number of new developments in GPC demonstrates the popularity and versatility of the technique, and bodes well for those looking to separate macromolecules on the basis of size in solution.

References

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Greg Saunders is Manager of the Polymer Characterization Technical Support Group at Polymer Laboratories, based in the UK. The team is responsible for the development of applications in the field of polymer chromatography, for providing technical support for PL's range of polymer characterization products and for providing training in chromatography theory and practice.