Gel Permeation Chromatography

Conventional versus multiple detection

Mark A. Jordi, Ph.D. and Maricel De Mesa, Ph.D.

Size exclusion chromatography (SEC), also known as gel permeation chromatography (GPC) or gel filtration chromatography (GFC), is a widely accepted analytical method used in the separation, purification and characterization of biopolymers and synthetic polymers. One of the primary uses of SEC is for the determination of polymer molecular weight. Currently, most GPC analyses are performed by comparing the molecular weight of a sample against standards of known molecular weight. This method is often described as classical GPC. A newer method is becoming increasingly common, which uses multiple detectors to provide absolute molecular weight information. In our experience, both methods have their own distinct advantages, and a thorough understanding of their strengths can aid in selecting the most beneficial method for a particular analysis.

Background

Unlike other chromatographic methods, SEC utilizes a non-interactive mode of separation. It employs a stationary phase composed of a macromolecular gel containing a porous network. As the polymer traverses the column containing the gel, the components of the sample are sieved based on differential pore permeation. Molecules with a hydrodynamic volume larger than the largest pores of the stationary phase cannot penetrate the pores of the gel, and then pass through the spaces between the gel particles unretarded. On the other hand, molecules with smaller hydrodynamic volume enter the pores and the open network of the gel, and are retained in the stationary phase to varying degrees, depending on their size and shape. This results in an elution order based on decreasing molecular size.

Through the years, SEC has gained popularity, not only because it can separate biomolecules such as proteins, enzymes, nucleic acids, polysaccharides and hormones, but also because it can be used to determine the molecular weight averages and molecular weight distribution of synthetic and naturally occurring polymers. The most widely used method in SEC is conventional GPC, which makes use of a single concentration detector, most often a differential refractive index (RI) or a UV spectrophotometric detector. RI detection is more universal than UV and has become the detector of choice for most GPC separations. Molecular weight determinations by conventional GPC, using either RI or UV detection, rely on comparison of the sample with standards of known molecular weight. In this method, a calibration curve (log Mw versus retention volume) is created, which allows determination of sample molecular weight based on the sample retention volume. The primary limitation of conventional GPC is that the molecular weights obtained are relative values. Thus, the accuracy of the method depends upon the standards and sample having the same relationship

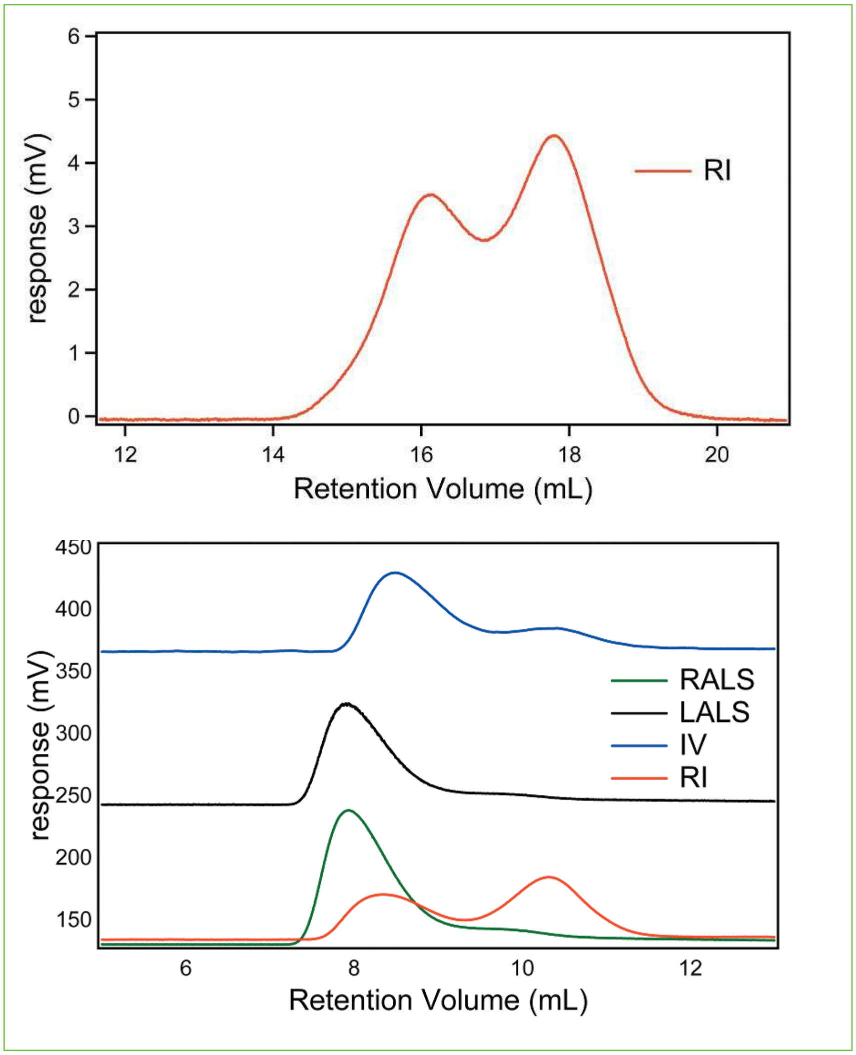

Figure 1: Classical GPC chromatogram (top) for a partially agglomerated sample and multidetector signal (bottom) for the same sample.

between their hydrodynamic volume and molecular weight.

In recent years, the use of multidetector GPC systems has become increasingly more common. These systems typically consist of multiangle light scattering coupled with refractive index and viscometry detection. These systems provide a significant increase in the amount of information that can be obtained during the GPC experiment. Parameters such as the radius of gyration, radius of hydration, intrinsic viscosity and the various molecular weight averages can all be obtained from a single experiment. Light scattering detectors measure the light scattered inelastically (i.e., Rayleigh scattering) and, with the use of the Zimm relationship Mw, this can be obtained directly. The viscosity detector measures the pressure drop and, in combination with a concentration detector, allows calculation of the intrinsic viscosity (inverse molecular density). This structural information can be used to probe such important features of the polymer system as its shape and branching characteristics.

Classical versus multidetector

Multidetector GPC provides significant advantages in terms of the information obtained and, based on this fact, one might assume that this technique is superior for all purposes. In our experience, both techniques have advantages, and classical GPC remains a viable alternative for many analyses. The primary factor that determines the best method is the purpose for the analysis. Most GPC analyses are performed for the purpose of determining the molecular weight, but the reasons for determining molecular weight are also varied. Some of the most common reasons to determine molecular weight include:

- theoretical studies of polymer systems,
- routine quality control,
- troubleshooting polymer failures, and
- regulatory concerns for polymer exemptions.

One of the clearest cases that can be made for multi-detection is for theoretical studies of polymers. Polymer research is a very exciting field that is exploring such fascinating topics as supramolecular chemistry, nanotechnology, controlled polymer architecture, biodegradable polymers and the mimicking of biological systems. Polymer systems of ever-increasing complexity are being produced. This array of new molecules often requires additional information beyond molecular weight. Furthermore, the choice of the standards used for a conventional GPC analysis has become increasingly important if accurate molecular weights are to be obtained. This is especially true for polymers with non-linear molecular architecture and for charged polymer systems. Polymer systems are now commonly being produced with non-linear geometries such as stars, combs, dendritic and hyperbranched materials. Even more mature polymer systems also have the possibility of containing short- or long-chain branching. In such cases, multidetector GPC offers clear advantages. Classical GPC provides molecular weight information solely based on hydrodynamic volume (molecular size). Two molecules with differing polymer architecture can have the same hydrodynamic volume but have very different molecular weights. Classical GPC cannot distinguish these cases. Light scattering detection coupled with viscometry provides the absolute molecular weight and the intrinsic viscosity. The Mark-Houwink plot can be accessed showing trends in chain branching as a function of molecular weight and the general shape of the molecule (rod, sphere, random coil) can be determined. The polymer size in solution can also be determined. Theoretical work clearly benefits from the increased information obtained using multidetector systems.

GPC is often used as a quality control measure for polymer production. This includes both product release testing as well as product failure analysis. Many of the most crucial properties of a polymer are dependent upon molecular weight, including strength, elongation and crucial processing parameters such as melt temperature and viscosity. Molecular weight determination is a good way to predict a polymer's behavior, or to determine why a material is not performing. Multidetection and classical GPC analysis both play an important role in serving this function. Some of the important considerations for any routine analysis include reproducibility, cost, instrument reliability, analysis time and the ability of the operator to competently interpret the data. The balance of these factors determines which technique is best for a particular application. Multidetector GPC is often the best choice for high-end applications such as drug delivery polymers. These systems tend to be more complex and include a more unique polymer architecture. In our experience, absolute molecular weight determination also has improved reproducibility for analyses that are ongoing over long periods of time.

A number of regulatory bodies currently offer exemptions for materials that meet the requirements.

Classical GPC offers the advantage of reduced cost both in terms of initial instrument setup and during ongoing maintenance. This latter cost should be considered carefully as the complexity of multidetector systems may require more routine maintenance than a classical GPC system. Without a well-implemented maintenance program, these same reliability issues can lead to increased downtime for the more complicated multidetector systems.

The final factor that should be considered is the complexity of the data interpretation. For more routine applications, it is sometimes the case that operators with limited experience in GPC may be performing the analysis. Interpretation of multidetector GPC results does require a reasonably high level of proficiency in order to accurately determine the meaning of the various signals. This is especially true in cases where the meaning is only clear when considering the relationship between the signals.

Analyses that are performed to satisfy regulatory requirements are an example of an analysis in which classical GPC is often preferred. A number of regulatory bodies currently offer exemptions for materials that meet the requirements.

This includes the EPA and the European REACH legislation. The classification as an exempt polymer is generally based on an analysis of the monomeric and oligomeric content of the material, among other things. In the case of REACH, the legislation exempts polymeric materials so long as the monomer does not make up more than 2 percent by weight of the final material. Multidetector systems are not suitable for these analyses, as molecules of under 2,000 molecular weight do not scatter sufficient light to be detected. This provides an artificially low estimate of the low molecular weight content. Multidetector systems will provide an indication that the oligomeric and monomeric materials are present due to the RI signal, but they will have to be operated in classical GPC mode to perform the weight percent determination. This concept is a general one and the analysis of oligomeric or other low-molecular-weight materials by GPC is often best done using the classical method.

Closing

Multidetector GPC analysis is an exciting tool providing the researcher with increased information over classical GPC methods. In spite of the increased information this technique offers, we believe that it is not superior in all applications. The best method for a particular application requires an understanding of the strengths and weaknesses of each technique. ■

Mark A. Jordi, Ph.D., is the Vice President, and Maricel De Mesa, Ph.D., is a Senior Scientist, both at Jordi FLP. They may be contacted at ChromatographyTechniques@advantagemedia.com.

LC Product News