Gel Permeation Chromatography (GPC) or Size Exclusion Chromatography (SEC)

By Impact Analytical

About Impact

Impact Analytical is a US based contract analytical testing laboratory that provides analytical support for five main sectors: specialty chemicals including polymers and silicon materials, medical devices, pharmaceuticals, bioanalytical and agricultural products. Impact Analytical is known as a problem-solving laboratory and can assist in R&D support, method development and validation, required regulatory testing, stability studies, and competitive analysis. Impact Analytical is ISO 9001:2015 certified, DEA licensed, FDA registered, cGMP and GLP compliant.

Size Exclusion Chromatography (SEC) is a non-interaction based separation mechanism most commonly used with large molecular weight or polymeric materials in which compounds are retained for different periods of time based upon their access to the porous structure of the chromatographic packing. Any other mechanism which is interfering with a purely sized based separation is undesirable. In its simplest terms, SEC can be considered to be a form of filtration. From this perspective, the most important parameter is the hydrodynamic volume of the sample as compared to the pore size of the chromatographic media. In addition to the sample size in solution, the chemistry of the sample also plays a crucial role as it governs the sample solubility and stability. It further plays a crucial role in any non-sized based retention mechanisms. A careful review of the chemistry of the sample is the first step in any good method development project.

To develop a valid method, it is therefore crucial to closely scrutinize the mechanism of the separation so that all non-sized based interactions can be eliminated. This is primarily done through the modification of two parameters:

1. Mobile Phase Composition
2. Column Chemistry

It is also important to consider the chemistry of the sample and especially its solubility. We have seen many cases, in which an irreproducible separation was caused by changes in the sample hydrodynamic volume over time. This can occur through phenomena such as sample aggregation, crosslinking, or precipitation.

The following is a list of the primary factors that must be considered when developing a GPC method:

1. Purpose for the Analysis
2. Mobile Phase Composition
3. Column Chemistry
4. Column Porosity
5. Flow Rate
6. Dissolution Conditions
7. Temperature
8. Sample Stability
9. Sample Concentration
10. Sample Standard Selection

In considering each of these factors, the following concepts should be considered:

Purpose for the Analysis

The goal of the analysis will determine the most appropriate instrumentation to apply. It will also mandate the level of precision which must be obtained. If the purpose is to compare various lots of material, then standardized GPC is often the best method. If a theoretical understanding of the molecular structure of the polymer is desired, more sophisticated instrumentation such as light scattering detectors are desirable.

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Mobile Phase Composition

The mobile phase selected for the analysis will ideally be a strong solvent for the polymer being analyzed (high solubility – swelled polymer coils). The sample must also have a greater affinity for the solvent than it has for the column packing. It is our general observation that a good mobile phase can often be selected simply by considering the molecular interactions between the sample and solvent while contrasting them with the interactions which can occur with the column phase.

Column Chemistry

The column is one of only three parameters which the chromatographer can vary to overcome non-size exclusion effects. Column chemistry is generally best selected such that strong interactions occur between the solvent and the column. It is our experience, that this is even more important than the interaction strength between the solvent and sample with the obvious requirement that the sample must be soluble in the mobile phase of interest.

Column Porosity

The porosity of the columns must cover the complete molecular weight range over which the sample is expected to elute. The polydispersity of the polymer sample will determine the width of the required pore size distribution. For unknown samples or for routine analysis, we generally recommend a mixed bed column. For small molecule and oligomer analysis (<5000 Mw) a 100Å column generally works best. In situations in which high resolution is required, single pore columns may be the best option. The primary limitation of using single pore column sets is a reduction in calibration linearity and column flexibility.

Flow Rate

The analysis flow rate can generally be varied over a narrow range without adversely affecting the calculated molecular weight values. Typical flow rates in SEC range from .5 – 1.5 ml/min with the most common flow rate being 1.0 ml/min. The primary time when flow rate should be carefully considered is when separating high molecular weight compounds. Polymers with molecular weights in excess of a million can become very shear sensitive and slower flow rates may be required to prevent sample degradation.

Dissolution Conditions

The conditions used to dissolve the sample can also play a crucial role in the analysis. In general, we have found it preferable to use a gentle shaking method for sample dissolution. Stirring with a magnetic stir bar is highly discouraged as many polymers degrade under such conditions. In addition, the temperature and time used for the dissolution should be considered. Many insoluble polymers can be dissolved successfully if gentle heating is applied. Polymer dissolution time varies and may take as long as 72 hours. It is important that full solubility is achieved for analysis. For highly crosslinked materials this may not be possible. During sample filtration prior to analysis it is important to observe any indications of increased backpressure. This may be an indication that the polymer is not fully dissolved.

Temperature

Temperature is the last of the three parameters which can be used to prevent non-size exclusion behavior. Increasing the column temperature can in some cases, prevent sample column interactions and result in an improved separation. In addition, the solvent viscosity decreases with temperature reducing column back pressure. In our experience it is generally preferable to run at an elevated temperature even when a lower temperature analysis could be performed. The main exception to this is when the polymer being analyzed is thermally labile or when the increased temperature may induce crosslinking of the sample.

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Sample Stability

There are a number of reasons why a sample may degrade during a GPC analysis. This includes shear degradation for high molecular weight polymers, hydrolytic degradation for polymers which degrade in the presence of water or thermal instability. Careful consideration should be given to the possibility of sample degradation when starting method development. In addition, some samples have the potential to crosslink and can increase in molecular weight over the course of an analysis. This may result in samples that become insoluble resulting in potential column damage.

Sample Concentration

The concentration of the sample can also have a large effect upon the quality of a GPC analysis. Typical GPC solution concentrations range from 1-4 mg/ml for random coil polymers of less than 800,000 molecular weight to .1-.5 mg/ml for high molecular weight polymers. Oligomers and monomerics can often be analyzed at higher concentrations of up to 10 mg/ml. The concentration is important because column overloading can result in degraded resolution and may introduce significant errors into the molecular weight calculations. In general, the lowest concentration which can be applied while obtaining good signal strength is the preferred concentration.

Sample Standard Selection

The geometry of the sample is an important consideration when choosing the type of standards to use during an analysis. Polymeric molecules may adopt a range of conformations including random coils, rigid rods, or spheres. In general, polymers will adopt a random coil configuration in solvents in which there are favorable interactions. Tightly balled coils can result if the polymer has poor solubility in the solvent system. Rigid rods are generally observed for helical polymers. The standard chosen for the analysis will ideally have the same chemistry as the samples being analyzed. In many cases, standards of the same type are not available. In these cases, the best standard is one which has the same geometry as the sample. Selecting a similar chemistry to the polymer being analyzed will tend to maximize the accuracy of the calculated molecular weights.

To choose initial starting conditions for the method development project, Impact may submit the following questions:

1. In what solvent(s) is the sample soluble?
2. Do you consider the sample to be polar, non-polar, or intermediate?
3. Does the sample contain ionic functional groups?
4. Do you have an estimated molecular weight range?

GPC/SEC is an excellent tool for understanding polymeric materials provided the correct conditions are chosen for analysis. A lab with experience and expertise is needed to provide accurate information on molecular weight and polydispersity.

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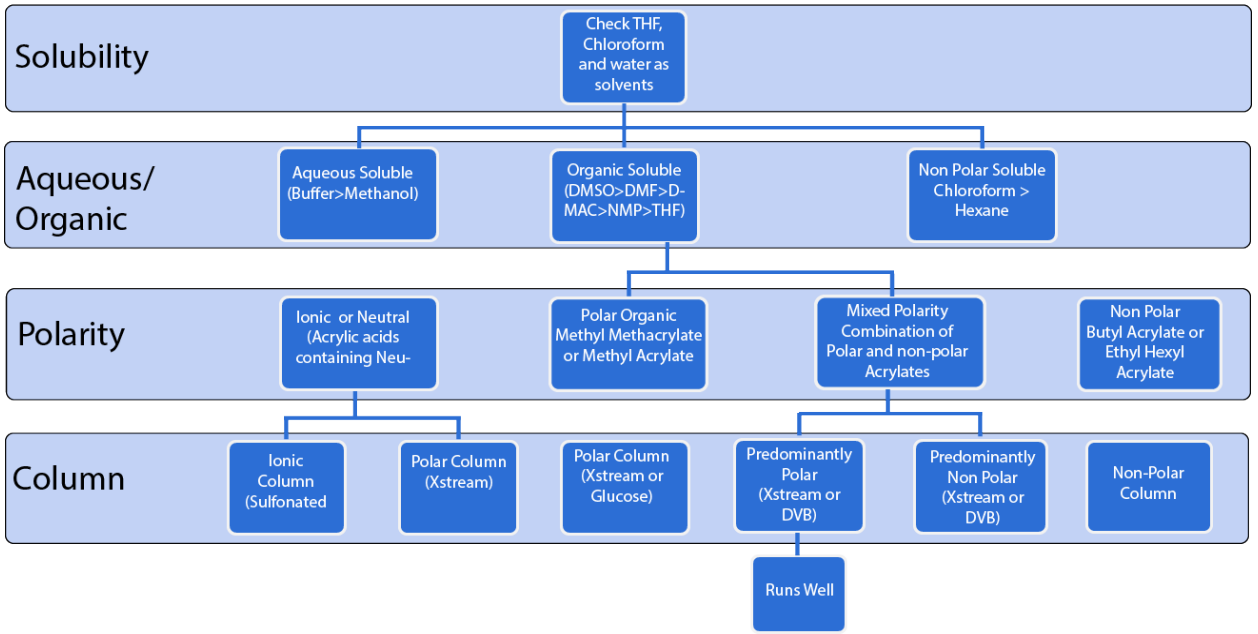
Decision Tree for GPC



Solvent Strength Chart

Water
Methanol
DMSO
DMF
DMAC
Acetone
NMP
THF
Toluene
Chloroform
Hexane