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

Size Exclusion Chromatography (SEC) is a non-interaction based separation mechanism 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.

In order 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. Column Chemistry
2. Mobile Phase Composition

It is also important to consider the nature 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 Geometry/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 viscometry and light scattering detectors are desirable.

Mobile Phase Composition

The solvent 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 500Å 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 molecular weight can become very shear sensitive and slower flow rates can be require 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 an orbital shaker 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. Most polymers are completely dissolved after a 12 hour dissolution period. In some cases this may be extended such that a polymer may require as long as 72 hours for complete dissolution. We recommend paying close attention during sample filtration prior to analysis to observe any indications of increased backpressure. This may be an indication that the polymer is not yet fully dissolved.

Temperature

Temperature is the last of the three parameters which can be used to prevent non-size exclusion behavior. Increasing the 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 room 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 cause crosslinking of the sample.

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 are crosslinkable and can increase in molecular weight over the course of an analysis. These samples may also 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 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 Geometry/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 or other rigid sample geometries. 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, we would submit the following questions:

1. In what solvents is the sample soluble?

2. Do you expect the sample to be polar, non-polar, or intermediate?

3. Does the sample contain ionic groups?

Figure I, on the next page, shows the progression of thought based upon the answers to these questions.

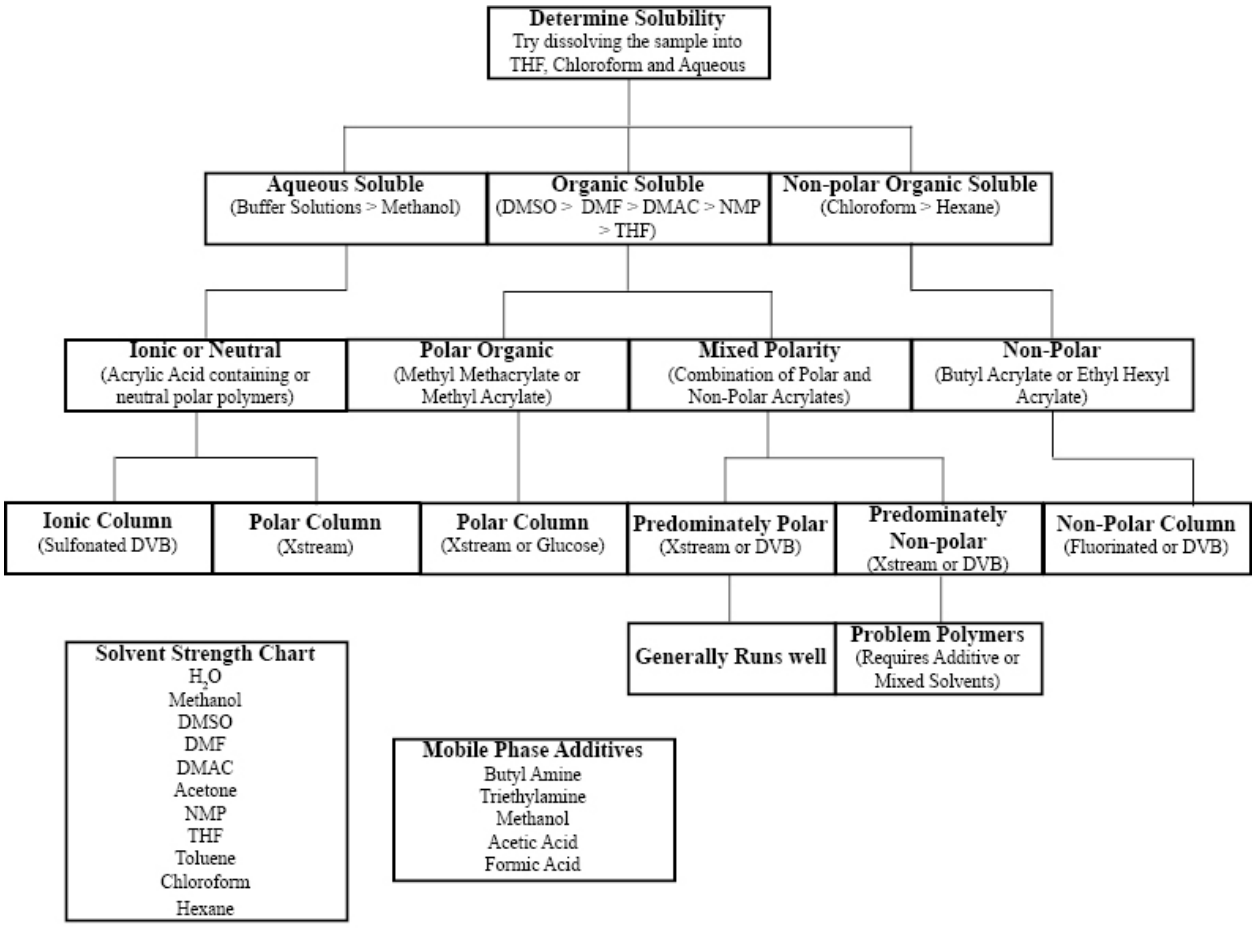

Figure I: Shows the progression of thought which can be followed to determine a logical column/solvent combination to use during an initial GPC method development effort.