## Application of GPC/SEC/GFC Standards and Polymeric Reference Materials

How to ensure the proper calibration of size-exclusion chromatography columns and detectors

## INTRODUCTION

Gel-permeation chromatography (GPC), size-exclusion chromatography (SEC), and gel-filtration chromatography (GFC) are chromatographic techniques used to determine key properties of macromolecules, such as molecular weight and molecular weight distribution. Analysts also use these standards to validate the performance of light-scattering detectors, viscometers, and mass spectrometers. GPC/SEC/GFC all work by separating and sorting the macromolecules according to their size by passing a solution of the macromolecules through a column packed with a porous material. These are relative techniques therefore to obtain molecular weight values, analysts need to calibrate the instrument and columns using rigorously characterized polymeric reference materials. The correct reference material to use depends not only on the type of macromolecule of interest and the range of its molecular weight, but also on the nature of the solvent used to dissolve it as well as the type of stationary phase in the column that will perform the separation.

## **IDENTIFYING SUITABLE REFERENCE MATERIALS**

The hydrodynamic volume of a polymer, i.e., its size in solution, is the key factor for determining how GPC/SEC/GFC achieves separation as the material passes through the column. The size in solution depends on the chemistry of the polymer, the type of solvent used, and the polymers' molar mass The same polymer will have a smaller volume in a poor solvent and a larger volume in a good solvent (FIGURE 1). A higher molecular weight polymer will have a larger hydrodynamic volume than one of low molecular weight, given they have the same composition. In addition, under comparable conditions, branched polymers have smaller hydrodynamic volumes than linear polymers. The result is that it is often possible for very different polymers to have identical hydrodynamic volumes, which could lead to erroneous calibrations if the reference material is not the correct one or if it is not specified with the application.



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For example, when dealing with polymers that involve branching, the reference material must have the same degree of branching as the materials of interest. If there are no comparable reference materials, one must perform additional analyses to determine the correct molar masses.

The choice of reference material will depend primarily on what the analyst needs to calibrate. Most commonly, GPC and SEC columns. First, the column's pore size distribution must be determined, which changes based on the range of molecular weights one is interested in. Then, to perform a calibration, analysts can use a set of narrow standards. This is typically eight to 12 samples of polymers of the same chemical type and structure, each with a narrow molecular weight distribution. The range of molecular weights in the kit should cover the entire range of the column. These standards are available as kits, supplied in common autosampler vials in a variety of chemistries. The kits include certificates of analysis (CoA) with all the necessary parameters to construct calibration curves, such as weightaverage molecular weight, number-average molecular weight, density, dispersity, and most importantly, the peak molecular weight M<sub>p</sub>.

PSS synthesizes and characterizes its reference materials by the strictest quality standards as detailed in ISO 9001. If the kit contains 12 standards, analysts must perform 12 runs to make a proper calibration curve. To shorten this process, PSS also offers standards in pre-configured mixtures of up to four standards in three vials, which take as little as a quarter of the time to run. For the most accurate results, calculate the sample volume by weighing in the eluent and using the provided density to convert mass to volume. This is especially important when calibrating instruments that use viscometers and light-scattering detectors.

# CALIBRATING COLUMNS AND DETECTORS WITH POLYMERIC REFERENCE MATERIALS

When calibrating a GPC system, the type of column will influence the peak shapes in the chromatogram. For example, when analysts ran a set of polyethylene glycol (PEG) standards on an SDV column with 5  $\mu$  particle size and porosities from 1,000 - 1 million Å, they obtained four well-resolved peaks. In contrast, when they used an SDV column with 3- $\mu$  particle size, there were three well-resolved peaks in the high molar mass end and a series of peaks in the lower mass end, indicative of oligomer separation (**FIGURE 2**). Using PEG's repeat unit

#### FIGURE 2: Influence of Column on the Calibration Curve

- The calibration curve describes the separation range of the GPC/SEC columns. It is governed by the pore size distribution of the columns.
- A more shallow slope indicates better separation power.
- · The calibration curve is almost always S-shaped, very rarely linear.

Example: Calibration curve and elugrams of the same standards mixture on two different SDV column sets:





molecular mass of 44 g/mol and a set of mono-dispersed PEG standards, they calculated the mass of each of the observed peaks and added them to the calibration curve. This technique can be applied to other polymers as well.

Not all polymers are available in narrow distribution form, however, because of their chemistry or the way they are produced. To prepare calibration curves using broadly distributed standards, analysts can use the GPC software to adjust a base calibration curve, assembled using narrow standards, to reflect the desired polymer. They must first create a base calibration using a set of narrowly distributed standards of polymers of a different type, such as PS or polymethylmethacrylate (PMMA). The calibration standards must match the mobile and stationary phases used in the instrument. The next step is to measure several broadly distributed standards of the desired polymer with a known weight-average molecular weight, number-average molecular weight, or intrinsic viscosity  $[\eta]$ . The software will then use these parameters to perform the necessary math to shift the calibration curve (FIGURE 3).

Analysts can also use these standards to calibrate a variety of detectors, including light-scattering detectors, concentration detectors, as well as viscometers. While they do not need to develop a calibration curve for the column in the case of light scattering, they will need several system parameters for this type of calibration. These parameters include inter-detector delays and detector constants. In addition, analysts must normalize any multi-angle light scattering (MALS) detectors. The information provided with narrowly distributed polymeric standards will allow them to get the most accurate measurements.

## PREPARING, HANDLING, AND STORING POLYMERIC REFERENCE MATERIALS

To prepare standards, first identify the mobile phase that works best for the application of interest. The ideal mobile phase allows for the dissolution of the entire sample without decomposition and allows the detection of the solute (FIGURE 4). When using a refractive index (RI) detector, the mobile phase should have a different RI than the solute to maximize the RI increment (dn/dc) and thus maximize the RI response. For example, tetrahydrofuran (THF) does not work

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with polydimethylsiloxane (PDMS) because it is isorefractive with it, making dn/dc equal to zero. This means that no matter how concentrated the sample solution is, the RI detector will show no response. In this case, toluene would be a better choice of eluent, since it is a good solvent for PDMS, does not cause its decomposition, and has a different RI. Analysts must make similar considerations when using UV detection, selecting a mobile phase with the appropriate UV absorption. The mobile phase must be pure and as free of dust and particles as possible and preferably non-toxic.

To identify the best stationary phase, analysts need to consider its polarity and that of all the components of the mobile phase. A graphic device available from PSS facilitates

#### FIGURE 3: Calibration with Broad distributed polymer standards



#### FIGURE 4: Selecting appropriate standards for GPC/SEC



Mobile phase considerations - the ideal mobile phase

- · Allows dissolution of entire sample without decomposition
- Allows detection
- Is free of dust, particles and other contaminants
- Is non-toxic, non-harmful, does not corrode the instrument
- ls a thermodynamically good solvent





this process. For example, if using styrene as the reference material, the preferred solvent should be somewhat polar, such as THF or toluene. According to the diagram in **FIGURE 5**, the appropriate column would be SDV. The next step is to determine the best concentration of the samples to avoid overloading the column. This concentration will depend on the molar mass of the standards, and PSS has specific recommendations for all its columns.

Another important aspect of preparing and handling the solutions is the time it takes for macromolecules to fully dissolve this can range from a few hours to a few days. Analysts should refrain from using ultrasonication, microwave ovens, or highspeed mixers to expedite this process, as they can degrade the polymers. Macromolecules require gentle treatment. Warming the samples is effective in some cases, such as with amylopectin in DMSO, but it can be detrimental in others, such as PEG in water. To verify that the polymer has dissolved completely, analysts should monitor the peak area, as dissolution will be complete when successive injections yield the same area. PSS discourages users from freezing or storing standards for a long time and encourages them to use freshly prepared samples. PSS' reference materials have an expiration date noted on each product. To illustrate this, PSS analysts ran a ReadyCal standard directly after making it and ran the same solution again two weeks later. They observed that the highest molecular weight disappeared, likely due to degradation and that the other peaks became broader for the same reason.

### CONCLUSION

Calibrating GPC/SEC/GFC systems requires the use of high-quality reference materials and strict adherence to recommended workflows. To ensure success, analysts should use kits of polymeric reference materials that contain not only carefully produced materials but all the necessary information for choosing the appropriate polymers and for constructing valid calibration curves. With proper preparation, handling, and storage, these materials enable analysts to carry out successful calibrations.