Step-by-Step Method Development for GPC/SEC

Technnical Overview

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Abstract

Agilent offers a full suite of GPC/SEC solutions for the many different applications and samples measured with this technique. Resilient methods and correct product choice directly impact a laboratory's budget by maximizing throughput and reducing data errors. This technical overview reviews each step of method development from initial compound identification to column end-of-life.

Introduction

Gel Permeation Chromatography (GPC), also called Size Exclusion Chromatography (SEC) or Gel Filtration Chromatography (GFC), is the primary method of determining a sample's molecular weight distribution.

GPC columns are filled with porous beads of a well-defined pore diameter and large pore volume. After injection, small molecules drift into the pores, while large molecules are excluded and get swept along by the solvent. This separation mechanism causes analytes to elute from largest to smallest.

Precise method development is necessary to ensure that data are consistent and error-free, while also minimizing the time spent developing each analysis.

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Getting Started

Identify the compound

Chemical identification is usually provided in the product documentation or the safety data sheet.

If nothing is known about the target sample, a spectroscopic method can be used to identify it. Figure 1 shows how an Agilent 4300 portable FTIR enables easy identification of polymers.

Figure 1. Agilent's 4300 Portable FTIR offers easy identification of polymers

Identify potential solvent-temperature-phase chemistry combinations

Before purchasing a column, refer to *Polymer-To-Solvent Reference Table for GPC/SEC* (5991-6802EN) [1], to identify the appropriate phase and solvent for your application.

Identify the molecular weight (MW) range

The MW range of the sample is critical to choosing a column.

Polymers

- MW ranges are often present in manufacturing documentation. Offline techniques such as light scattering, rheology, and titration can be used for MW estimates.
- If only a single, average MW value is given, then an upper limit of 10x the average MW and a lower limit of 0.01x the average can be used.

Additives, prepolymers, and plasticizers

- Most nonpolymeric additives have an MW below 1,500 Da. Prepolymers and large MW additives normally fall below 10 kDa.

Sample cleanup

- In mass spectrometry, large molecules fragment during ionization and cause interferences. The exact MW limit varies by the ionization technique.
- To eliminate interferences from fats, waxes, and other large molecules during pesticide testing, EPA method 3640A uses an upper MW cutoff of 390 Da.
- For more information, see 5991-5321EN [2].

Review instrumentation

Temperature

- Verify that the sample is soluble at all the temperatures it encounters as it travels through the system (assume that exposed capillaries are at room temperature).
- For samples requiring elevated temperatures to remain solvated, the Agilent PL-220 High Temperature GPC/SEC system (G7820A) offers temperature control from dissolution to detection.

Pressure

- Verify that the pressure on the system does not exceed the limits of the pump or the column, as found in the user manuals.

Detector

Refractive index (RI)

- Most common in GPC
- Offers universal detection, but reduced sensitivity compared to UV-Vis
- Response is proportional to difference in refractive index between sample and solvent

UV-visible (UV-Vis)

- Common, sensitive detector
- Requires a chromophore on the sample and noninterfering solvents

Evaporating light scattering detector (ELSD)

- Higher sensitivity than RI
- Offers universal detection
- Sensitivity and response drop rapidly with volatile samples

Triple detection (combined refractive index, light scattering, and viscometry)

- Necessary to characterize complex samples, nonlinear/branched polymers, graft polymers, and copolymers
- In-line measurement of refractive index, intrinsic viscosity, and degree of branching

For more information, refer to: *A Guide to Multi-detector Gel Permeation Chromatography* (5990-7196EN) [3]

Dispersion

- Dispersion limits the maximum efficiency and speed that a system can achieve.
- High dispersion systems will have longer runtimes and lower resolution, and cannot be improved by simply switching to a higher efficiency column.
- Common sources of dispersion are capillaries, columns, fittings, flowcells, injection loops, and system dead volume.
- Steps can be taken to improve performance on both high and low dispersion systems. Refer to *Instrument Setup for Fast GPC* (5991-7191EN) [4].

Developing the method

Choosing a column

Select the appropriate column phase and solvent for your application from *Polymer-To-Solvent Reference Table for GPC/SEC* (5991-6802EN) [1]. Identify all columns that would be able to separate the compound based on their MW ranges from the brochure.

To determine which column will give the best performance, compare the efficiency and the calibration curve.

A column should elute the largest compounds as early as possible, and the smallest as late as possible to maximize resolution. The MW range should capture the target's MW range with as little excess as possible. Unimportant regions can go beyond the MW range, but the peaks will be highly distorted.

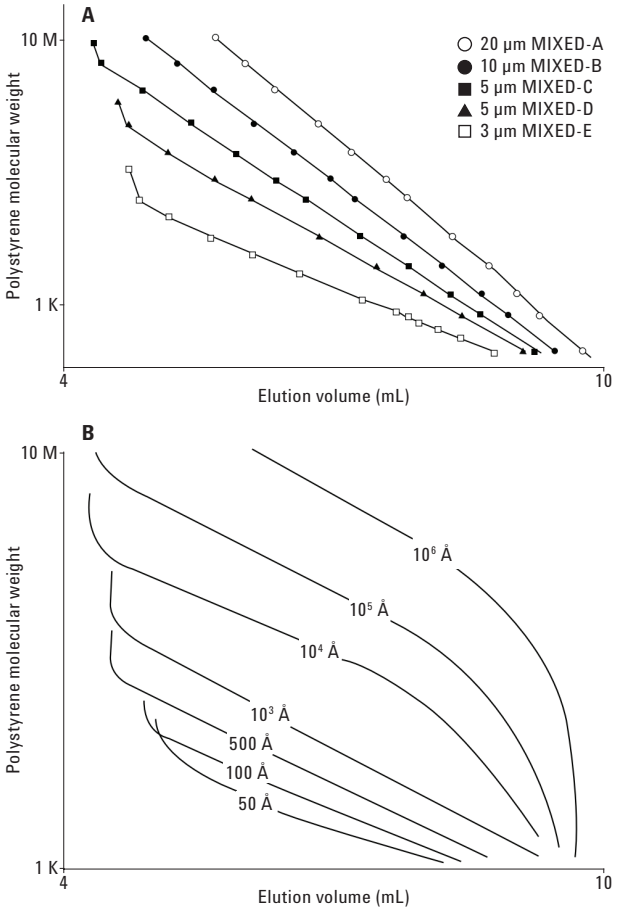

Figure 2. Calibration curves for PLgel MIXED and individual pore size columns.

If the final choice is between a column with better separation or higher efficiencies, then better separation is preferable. Efficiency is more easily lost to system dispersion or column degradation, while good separation is only impacted by the total length of column on the system.

Conditions

Solvent

A guide to most common solvent systems can be found in *Polymer-To-Solvent Reference Table for GPC/SEC* (5991-6802EN) [1].

- When working with uncharacterized compounds, it is important to verify that they are not interacting with the media.
- The calculated MW should be compared to a value found by a separate method, such as light scattering, titration, or viscometry.

Another sign of interaction is a peak order that does not correlate directly to MW, for instance, when a peak elutes beyond the low MW limit of the column (such as Figure 3), or when a known low MW compound elutes after a known high MW compound.

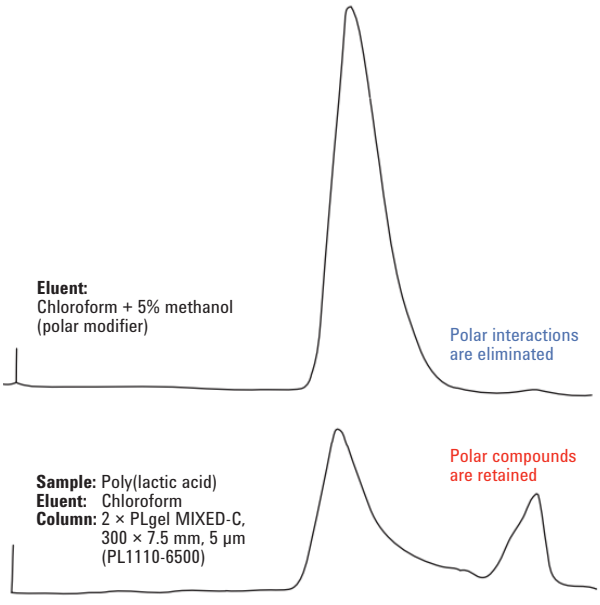

Figure 3. Polar interactions in the lower chromatogram are eliminated with a 5% methanol addition to the eluent.

Temperature

Elevating column temperatures, even by 5–10 °C, is highly desirable in GPC, as it reduces viscosity and allows higher efficiencies, lower pressures, and faster flowrates.

Flowrate

In GPC, flowrate must be optimized according to the sample. The relationship between solvent velocity and efficiency (van Deemter curve) varies with both particle size and sample MW.

Larger molecules require large particles, and resolve better at slower flowrates (SI-01745) [5]. Smaller molecules run best on smaller diameter particles, which have higher efficiencies, and operate best at higher flowrates (5990-8332EN) [6].

The flowrate is optimized by repeatedly injecting MW standards at different flowrates. The optimum flowrate is the one in which the standard peak closest to the target MW is narrowest and most well resolved. (SI-01745) [5].

Finally, wide diameter columns with higher total flowrates will offer superior efficiency and resolution on high dispersion systems. This strategy is used with the Agilent PL Rapide columns to maximize performance on systems with high dispersion [4].

Injection volume

Even at low concentrations, long polymer chains can drastically increase the viscosity of the solvent. Sample concentrations should be kept as low as possible. Multiple injection volumes and concentrations should be tested to identify the conditions that maximize the signal without altering the MW measurements. More details can be found in *GPC/SEC Column User Guide* (5991-3792EN) [7].

Software parameters

Excluding a peak's front or tail from the peak integration can cause significant variability in calculated values of Mn, Mz, and MW, which are derived from the statistical analysis of the total peak shape. Inconsistent values have little usefulness in predicting the polymer's physical and chemical properties.

In situations where the front or tail of a peak is missing, or a peak has an unusual shape (such as a bimodal peak), a direct overlay of calculated MW distributions is the best way to identify differences between samples.

Required Maintenance

Monitoring column status

Calibration: Columns must be regularly calibrated with standards to obtain accurate MW values. Error increases exponentially in GPC/SEC, so a 1% inaccuracy in retention time can easily cause a 10% shift in measured MW.

Pump drift, column aging, new capillaries, reswaged fittings, and new column connections all cause retention times to shift. Systems calibration before and after data collection eliminates these errors. For more information, refer to:

- *Calibrating GPC Columns, A Guide to Best Practice* (5991-2720EN) [8]
- *Agilent Standards Brochure* (5990-7996EN) [9]

Flowrate marker: To mark the end of the run, a small molecule such as toluene, BHT, acetone, or ethylene glycol, is added to all samples as a flowrate marker. The narrow peak from the marker (Figure 4, peak 6) can be used to measure column efficiency and identify degradation, tailing, and retention shifts that are not obvious with broad sample peaks.

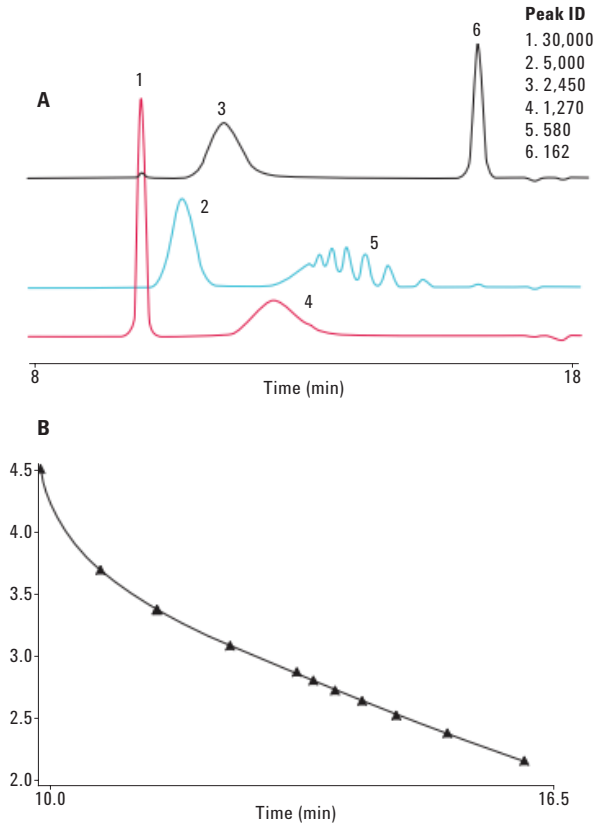

Figure 4. Calibration of a small pore size column.

Troubleshooting

Agilent GPC/SEC columns set the standard for ruggedness and lifetime, but do eventually degrade.

Some failure modes are unique to GPC/SEC, and familiarity with these modes of failure enables investigators to avoid generating inaccurate data:

- **Large pore collapse** – Flowing solvent slowly crushes the largest pores, so that large molecules are no longer separated.
  - **Indication**: High MW standards are not separated. At the beginning of the chromatogram, the largest molecules elute all at once, and appear as a peak or shoulder.
  - **Solution**: Regular calibration tracks pore collapse, and column replacement improves data quality.
- **Bed degradation** – Particles slowly crush with use, which shifts the pore size distribution, and reduces overall separation and efficiency.
  - **Indication**: Loss of efficiency and less separation between the highest and lowest MW calibration standards
  - **Solution**: Track separation and efficiency loss with regular calibration and use of a flowrate marker. Replace columns when the efficiency drops below acceptable levels (usually 80% of the original value).
- **Chemical attack** – Reactive compounds can alter the surface of the particles and cause the analyte to stick. Common culprits are residual oxidizers, radical initiators, reactive prepolymers (such as acyl chlorides), and solvent degradation products.
  - **Indication**: Over time, the same samples become more retained, and peaks start to split or tail.
  - **Solution**: Before injection, neutralize any strong acids or bases, use BHT to deactivate residual oxidizers and radical initiators, and use alcohols to destroy any strong nucleophiles and electrophiles.

Conclusion

A well-developed GPC/SEC method allows analysts to maximize their laboratory's throughput and efficiency, while avoiding delays and errors.

Agilent is proud to offer a complete, industry-leading solution for GPC/SEC analysis as well as the expertise necessary to develop the best method for any analysis.

References

1. Adam Bivens. *Polymer-to-Solvent Reference Table for GPC/SEC*; Technical overview, Agilent Technologies, Inc. Publication number 5991-6802EN, **2016**.
2. Catherine Jones, Praveen Kutty, Alan Brookes. *An Automated System for the Routine Clean-up of Environmental Samples Prior to Instrument Analysis*; Application note, Agilent Technologies, Inc. Publication number 5991-5321EN, **2014**.
3. *A guide to multi-detector gel permeation chromatography*; Agilent Technologies, Inc. Publication number 5990-7196EN, **2012**.
4. Adam Bivens. *Instrument Setup for Fast GPC*; Technical overview, Agilent Technologies, Inc. Publication number 5991-7191EN, **2016**.
5. *Plate Height vs Flow Rate - Effect of Molecular Weight*; Technical overview, Agilent Technologies, Inc. Publication number SI-01745, **2010**.

For More Information

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