# Tips & Tricks GPC/SEC:
# Mobile Phase Considerations

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**There are many sources of advice on how to select the most appropriate stationary phase for sample analysis, but the mobile phase is not often discussed. The mobile phase is an essential and integral part of an analytical system and influences the success of the analysis and the quality of the data and results. Correct solvent preparation can save vast amounts of time that would otherwise be used for troubleshooting issues.**

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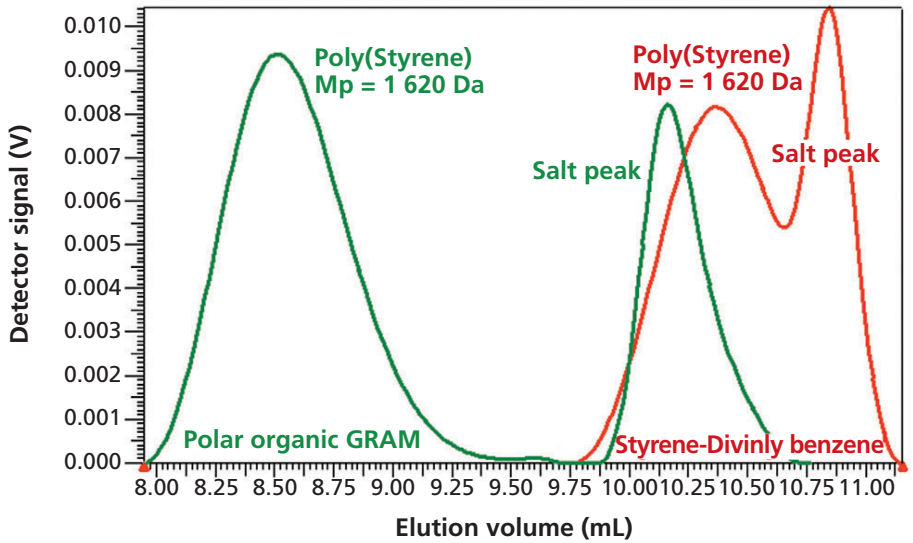
The choice of mobile phases available for gel permeation/size-exclusion chromatography (GPC/SEC) are limited to those that are compatible with the stationary phase and can dissolve the sample. Typically, GPC/SEC mobile phases are either aqueous (water, solutions of neutral inorganic salts, buffer solutions) or organic (tetrahydrofuran [THF], toluene, dimethylacetamide [DMAc], dimethylformamide [DMF], trichloromethane, hexafluoroisopropanol [HFIP], trichlorobenzene). The wrong choice of mobile phase can destroy columns with stationary phases based on cross-linked polymers, such as the most common organic stationary phase styrene-divinyl benzene, or hydrophilic polymer gels because they can shrink the swollen gel. Therefore, aqueous solvents should only be used with aqueous polymeric stationary phases and organic solvents should only be used with organic polymeric stationary phases.

Even if a solvent is classed as generally applicable with the stationary phase, it may not be sufficient to be used as is. Insufficient suppressing of the sample-stationary phase interaction often requires the addition of low molar mass modifiers or salts: Polar organic solvents, such as DMAc or DMF often need additives such as lithium bromide (LiBr) or lithium chloride (LiCl); and aqueous systems require the addition of additives to prevent algae growth and salts to suppress interaction.

Sometimes however, even with additives, a true size-separation cannot be achieved if the polarity of the three involved chemicals (sample – mobile phase – stationary phase) does not match. An example of this is the separation of poly(styrene) (PS) on styrene–divinyl benzene material in polar organic solvents, such as DMF or DMAc. Here, PS oligomers will elute with or after the salt peak (Figure 1). Therefore, either a polarity matched stationary phase should be used or poly(methyl methacrylate) (PMMA) should be used to calibrate the system.[1]

It is also important to ensure that the solvent allows the sample to be detected. In the case of refractive index detection (RI), the mobile

**Figure 1:** Elution of poly(styrene) (PS) 1620 Da in DMAc on a PS-Divinylbenzene stationary phase (red) and on a medium polar GRAM stationary phase. Lower PS oligomers will elute even after the salt peak on PS-Divinylbenzene.



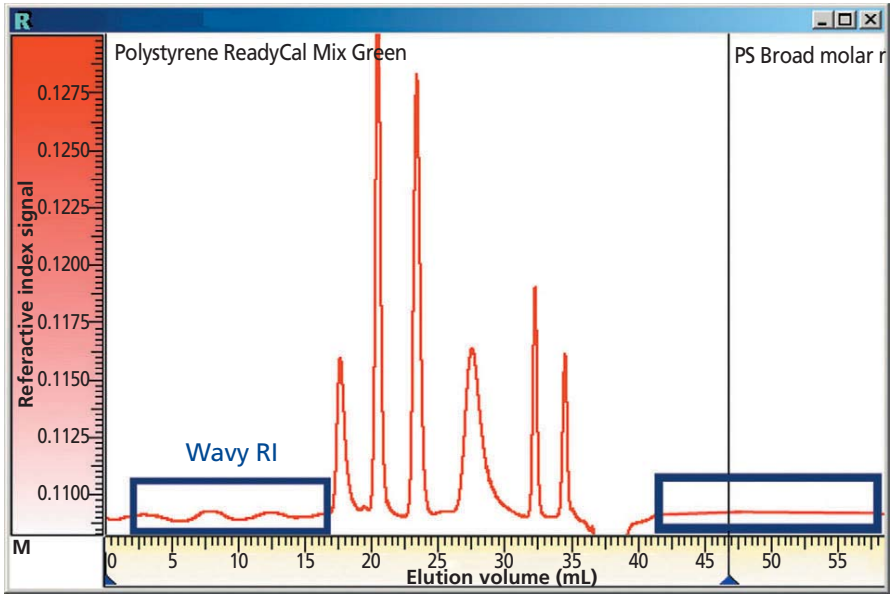

phase and sample refractive indices need to be as different as possible. If the difference is too small the signal intensity will be low. A famous example of an isorefractive system (refractive index increment dn/dc = 0) is poly(dimethylsiloxane) in THF. Although the sample is fully dissolved, it cannot be measured because of a missing detector response. It is therefore necessary to change the mobile phase to toluene (dn/dc < 0) or to use another detector such as an evaporative light scattering detector (ELSD).

Another factor to consider is solvent viscosity. Low viscosity gives increased resolution, so for highly viscous mobile phases (such as DMAc and DMF), elevated temperatures (60–80 °C) help to reduce the viscosity and therefore increase the resolution.

## Solvent Quality and Solvent Preparation

Although more expensive, solvents should be of the highest quality (HPLC-grade [high performance liquid chromatography]) as the

**Figure 2:** Wavy refractive index (RI) baseline as a result of insufficient eluent quality.




difference in purity is marked. All mobile phases and buffers should be prepared freshly on the day required. This will ensure that the buffer pH is unaffected by prolonged storage and that there is no microbial growth present as both could affect the chromatographic results.

Isocratic GPC/SEC chromatography requires a homogeneous mobile phase. Special attention must be given to solvent mixtures or solvents spiked with salts or other modifiers. If there is a large density difference between mobile phases in a mixture, or if the salt is not fully dissolved, chromatograms at the beginning and the end of a run might be shifted because of different solvent quality during the sequence.

Polar organic solvents are one such example. When using DMAc or DMF, Li salts are often used as additives. Careful preparation of the mobile phase is required because of the reduced solubility of these salts under these conditions. LiBr has the advantage of being more soluble than LiCl, but still requires heating to approximately 50 °C and stirring for at least 2 h.

Chromatograms might also shift if the water content in an organic solvent increases with time or if the solvent quality changes from oxidation, for example. THF should therefore be stabilized with a small amount of butylated hydroxytoluene (BHT) and chloroform with ethanol. Sodium azide ($NaN_3$) or similar should be added to aqueous solutions to prevent algae growth.

Before using the freshly prepared mobile phase, it should be thoroughly degassed to remove dissolved gas and avoid problems such as noisy or wavy baselines or spikes. The most commonly used method to degas is the use of an on-line degasser installed between mobile phase reservoir and pump. Care should be taken if HPLC degassers are used with organic GPC/SEC solvents because most of these degassers are not compatible with all solvents. It is also important that the mobile phase is free of small particles and dust that might cause blockages in the system or the columns.

GPC/SEC mobile phases should be exchanged regularly. Performing an analysis with solutions that are several days (or even weeks) old and have been run in recycle mode will most probably produce low quality data with drifting and wavy baselines (Figure 2). Operation under these conditions can even cause problems with pumps, injection systems, and detectors.

It should also be noted that the use of solvent plus salts requires special care for the instrumentation. Always apply a low flow-rate if the system is not in use to prevent corrosion of the instrument or the columns. Remove all salt solutions with pure solvents before exchange from one solvent to another, before you turn off the pump, or before you store a column.

## Summary

- The mobile phase is an integral part of the system and needs to be selected carefully.
- In the case of polymeric (cross-linked) stationary phases the wrong type of solvent (organic on aqueous gels or water on organic gels) can destroy the column, if the mobile phase collapses the gel structure.
- The use of high-quality (HPLC-grade) solvents, freshly prepared and degassed, can save time otherwise spent with troubleshooting issues and increases the quality of the results.

## Reference

1. T. Hofe and G. Reinhold, *The Column* **3**(12), 30–33 (2007).

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