

Tips & Tricks: GPC/SEC

Sample Preparation

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Polymers are sensitive molecules. Depending on the polymer architecture or copolymer composition, it is possible to have bonds present along the polymer chain that can easily be broken. In polystyrene solutions, breaking the polymer chains can happen, just by stirring a high molecular weight viscous solution.

Degradation and Chemical Reactions

The chosen solvent can also break up the dissolved polymer molecule. In this way, pullulans that are left to stand in a 0.05% sodium azide solution will break up within one to two weeks. The higher the molecular weight, the more visible this break up becomes (Figure 1 and Figure 2). If a pH smaller than 6–7 is used, this tendency will be accelerated.

Pullulan is not the only polymer that undergoes a change when stored in solvents. Above all, the water content in organic solvents and the oxygen content play an important role. Excluding oxygen during the sample preparation is virtually impossible or can only be achieved with great difficulty. Oxygen as a reactive radical, will attack polydienes (for example) and lead to chain extension,

branching and microgel formation. Although not as strongly demonstrated, this can also not be ruled out, for example, with polyacrylates.

For interaction free chromatography, additives such as acids, amines, salts and similar compounds should be used with the eluent and it must be ascertained that there is no reaction with the polymer under investigation. It is also not impossible to have chemical reactions during the analysis. The sample should be checked to ensure it is stable in the solution for enough time to undertake the GPC/SEC analysis.

Mechanical and Temperature Effects

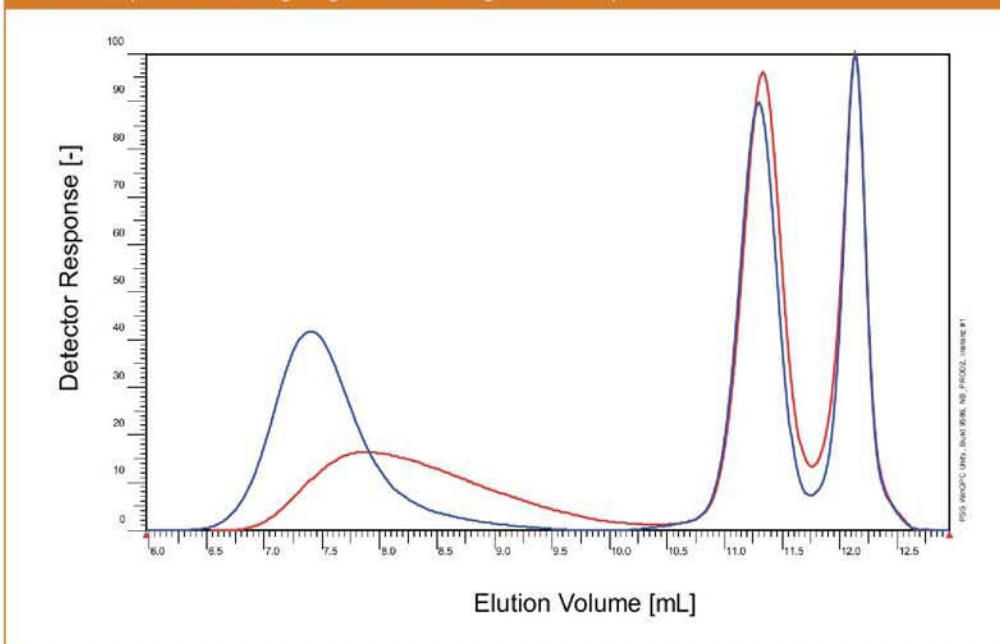
Every chemist or chromatographer wishes to generate results as quickly as possible. However, speed is not appropriate when analysing polymers. Processes to dissolve the sample quickly, that can often be used without any problems for small molecules, may only damage the macromolecule when applied to polymers. That is particularly true of exposure to microwaves and is also not advisable with ultrasound. Higher temperatures are only helpful when the polymer has increased solubility



at higher temperature although this isn't the case for polyethylene oxide and polyethylene glycol in water and also a whole range of modified celluloses that fall out of solution at higher temperatures (Figure 3). High temperatures are also prohibited in the case of thermally labile bonds. On top of these phenomena, there is also the alteration of the helical structure in biopolymers that can be evoked by a change in temperature or pH.

Should it be necessary to measure globular proteins, care is also required at higher temperatures if the globular structure is to remain intact. The choice of eluent is also important to prevent denaturing of the globular protein (Figure 4). In other words, the intermolecular interactions should remain intact. With proteins in particular, it is often necessary to measure dimers and higher oligomers. There are a whole host of

Figure 1: Pullulan degradation. Blue line: Fresh Pullulan solution. Red line: Pullulan solution after initial period showing degradation of high Mw component.



intermolecular reactions that, if not disrupted, will result in aggregates being seen in the chromatogram. If disulphide bridges exist in the protein it must be established whether these should remain intact, if one wants to measure the dimers, or be dissolved if the goal is to measure the single chain.

Normally, intermolecular hydrophobic or hydrophilic interactions should be suppressed to measure the true molecular weight

distribution. If this does not happen then either the sample will not completely dissolve or they could appear as aggregates in the chromatogram. An example is a PVC that contains an amount of syndiotactic PVC. If this product were to be measured then one must dissolve the PVC in either THF in a pressure vessel, or in cyclohexanone, which, without the pressure vessel, means that high dissolution temperatures must be used.

Complex and Laborious Preparations

Aggregate formation can be reversible. When left to stand, the solution can also form aggregates again. Dextrans and pullulans are clearly soluble in pure water but, when analysed in pure water, show aggregates as well that show up as multimodal distributions in the high molecular weight region. The addition of a small amount of a salt (e.g., 0.05% sodium azide) destroys the aggregates

and the dextrans and pullulans can now be measured without problem.

Many methods for sample preparation are elaborate and time consuming. For natural starches, procedures are described using water under high pressure at high temperature or a DMSO solution is made that is stirred for 24 hours under nitrogen at 80 °C. In some cases it is necessary to stir for a further 5 hours at 150 °C (Figure 5).

Figure 2: Pullulan degradation. Blue line: Fresh Pullulan solution. Red line: Pullulan solution after longer period showing complete disappearance of high Mw component.

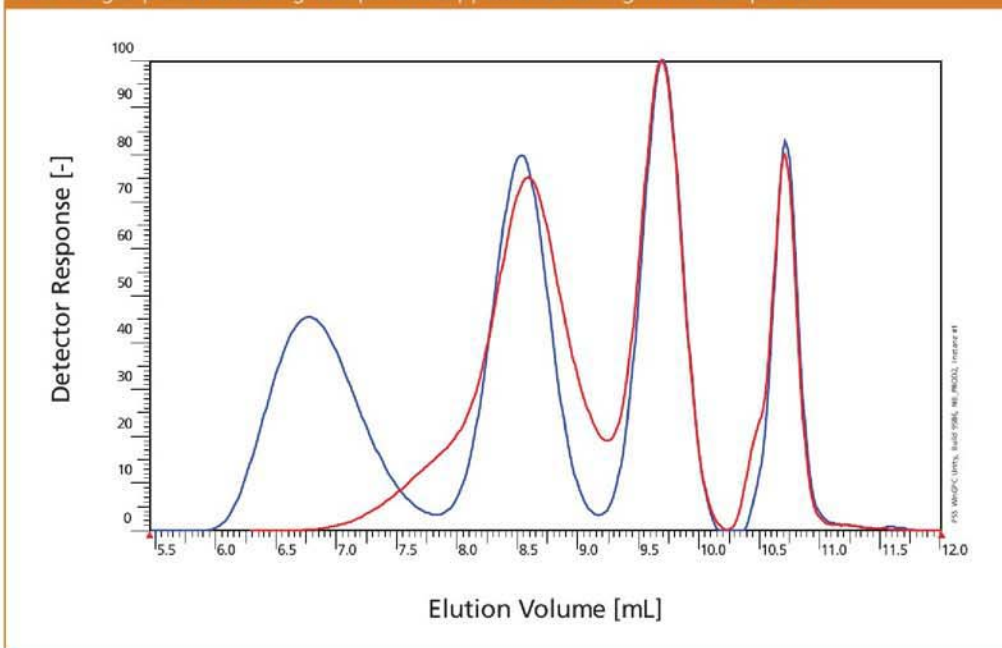
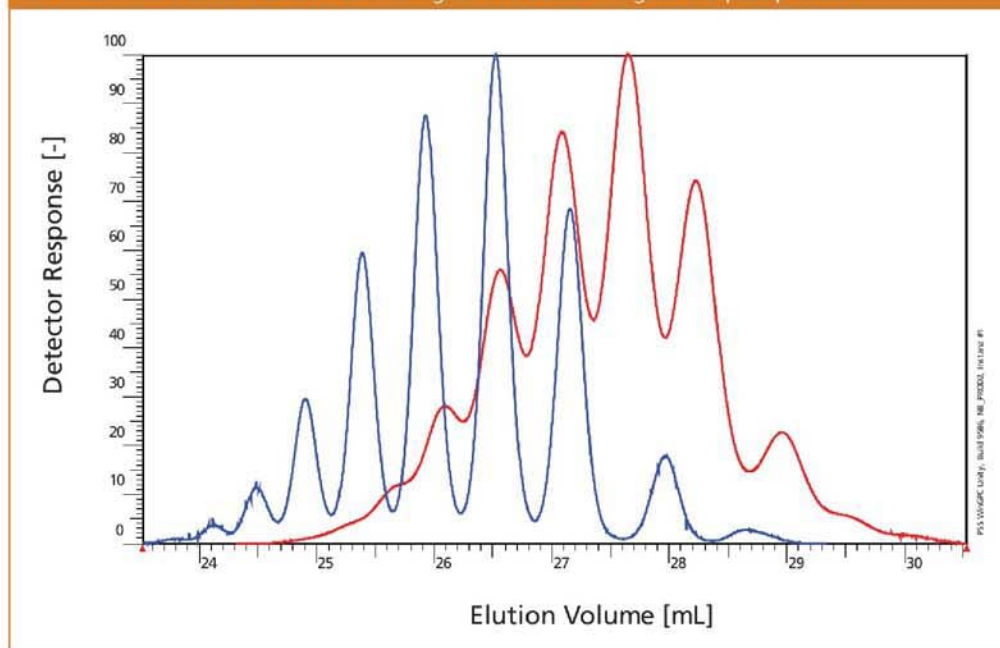


Figure 3: Effect of temperature on polyethylene oxide. Blue line: Measurement at 25 °C; Red line: Measurement at 80 °C. The higher molecular weights are precipitated.

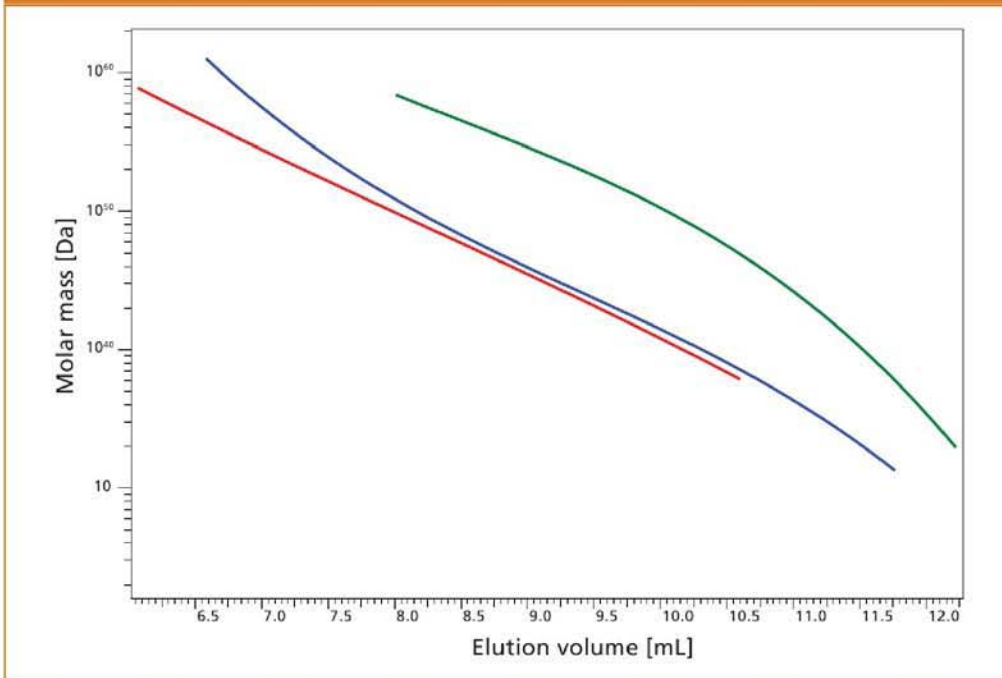


For cellulose samples, it is suggested to first swell in water and then afterwards transfer into acetone and finally to dissolve in 9% DMAC. Once the cellulose sample is dissolved it can be diluted with DMAC and then measured in a 0.09% solution. With all of these extensive procedures one must follow the method of sample procedure fastidiously to obtain

reproducible results. Whether one obtains the original molecular weight distribution through these sample preparation methods is difficult to estimate.

Also the possibility to derivatize polymers that are difficult to dissolve to achieve solubility in more common solvents is not without problems. According to reaction conditions, a degradation

Figure 4: Different calibration curves for proteins resulting from different sample preparation. Blue line: native proteins only dissolved in eluent; Red line: proteins heated at 50 °C; Green line: dissolved proteins measured in water/methanol mixtures.



of the polymer can occur. Aside from which, derivatization reactions on polymers never reach completion and 100% conversion is never achieved. Thus, derivatized starches and celluloses are always copolymers with more or less large differences in the sequence length distribution of the differently modified saccharide building blocks. This is sufficient for a relative

measurement using GPC/SEC. The use of a light-scattering detector requires an elaborate sequence of measurements in different solvents to obtain the true molecular mass.

Conclusion

Size exclusion chromatography is generally practiced as an isocratic technique. Therefore

the eluent should have as simple a composition as possible. High salt concentrations >> than 0.5 M or solvent mixtures can lead to fluctuations in the concentration in the eluent supply and thereby cause a poor signal-to-noise ratio. It is, therefore, always advisable to use a pure solvent (with water an anti-bacterial additive should be added to suppress, amongst other things, the build-up of algae, for example sodium azide). The use of modifiers such as acids, amines or salts in eluents, which often enable an interaction free elution, are only used in quantities that are just sufficient.

It should also be noted that with polyelectrolytes, the level of salt concentration alters the hydrodynamic volume and therefore, gives relative measurements in comparison with a calibration created with non-charged molecules, and leads to different results in the molecular weight distribution. High concentrations of additives or salts in the samples lead to extremely large system peaks (for example the elution of salts from the sample), which under certain circumstances make a reliable molecular weight measurement either difficult or totally impossible. The motto is use an eluent as pure and simple as possible.

References

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Figure 5: Sample preparation of starches. Blue line: 25 °C, then heated up for a short period of time to 50 °C; Red line: 24 hours at 80 °C. Amylose sample gives the same elution profile. Amylopectin gives different elution profiles because the higher molecular weight part only goes into solution at 80 °C.

