Tips & Tricks GPC/SEC: Answering Common Questions About GPC/SEC Columns

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The hardest part of any gel permeation chromatography/size-exclusion chromatography (GPC/SEC) separation is selecting the right columns and developing a robust method. Here, we present a selection of commonly asked questions from users together with our answers based on our experiences.

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is performed to determine the complete molar mass distribution. It can be applied over a wide range of molar masses for different types of natural and synthetic macromolecules soluble in mobile phases of very different polarities. GPC/SEC is often used in quality control (QC), but developing a robust and high-resolution method that delivers precise results, which are reproducible in the long-term, is a challenging task. It is therefore of no surprise that many users need expert advice when making the choice of the optimum column (set) from the large selection available. At a recent event we were asked a lot of good questions that we want to share here.

Q. When avoiding high backpressure and shear is it sufficient to run the GPC/SEC at a lower flow-rate (so long as you have enough time) or is larger particle size the better choice?

A: Macromolecules can be very sensitive, so forcing high molar mass or stiff polymer chains through a liquid chromatography (LC) system at a very high pressure can result in chain degradation and generate results only for the fragments. The overall pressure in a system depends mainly on the flow-rate, mobile-phase viscosity, temperature, inner diameter, number of columns applied, and particle size of the column stationary phase.

Small particles should be avoided when analyzing high molecular weights.[1] It is recommended to use larger particle sizes when running very high molar mass samples because this will reduce shear. Also note that in this case column frits with larger porosity are used and this further reduces shear stress. For really high molar masses a combination of both large particle sizes and low flow-rate is ideal, if time permits. If you are using highly viscous solvents, running at higher temperatures (to reduce mobile phase viscosity) is also recommended.

For lower molar mass samples, where high resolution is required (for example to separate oligomers) the application of larger particles to overcome back pressure issues is not recommended. Larger particles result in lower plate counts, thereby reducing resolution. Nevertheless most small molar mass samples will also profit from higher temperatures and lower flow rates for highly

viscous solvents because both approaches increase resolution as a result of better mass transport.

Q. Could you please comment on the pros and cons of mixed bed column vs. individual pore size columns?

A: This is a tough question because philosophy plays a part here and there are many aspects to consider.

Let us start with the often-mentioned expectation of a "linear calibration curve". Many people seem to feel more comfortable with linear relations, even though the requirements for simple mathematics has diminished with the widespread use of computers in the laboratory that can handle more complex algorithms.

Unfortunately, the relation between the logarithm of the molar mass and the elution volume is not linear. GPC/SEC calibration curves are typically sigmoidal in shape, where the logarithm of the molar mass is plotted versus the elution volume. Most of the time polynomial functions of 3rd (cubic), 5th, or 7th order are used to fit the data. This is a good approach as long as the slope of the calibration curve is also reviewed to avoid overfitting.[2] The approach also allows the full use of the complete separation range of the column. Please note that linear columns are also non-linear at the high and low molar mass end, therefore a different fitting approach is required if samples elute in that region.

Linear or mixed-bed columns are the result of intense work by column manufacturers. The production involves either a special synthesis route or, much more often, the careful blending of individual pore sizes. The main advantage of linear columns is that they can separate over a wide molar mass range with a constant resolution, and are ideal for routine QC or as screening columns if users have to deal with very different molar masses. You can easily increase the resolution by adding other linear columns of the same type. However, it is very difficult to alter the molar mass separation range when higher or lower molar masses need to be separated. The risk of porosity mismatch is extremely high, for example when combining linear columns with individual columns ideally suited for oligomer separations.[3]

The main advantage of individual pore-size columns is that they provide a highly efficient separation but in a limited molar mass range. Individual pore size columns are therefore often combined in column banks. Columns can be added and removed to alter the molecular weight to tailor it to the application and the time requirement.

When following this approach an additional advantage is that the column with the largest porosity can be used first to separate the high molar mass (and therefore high viscous) chains to avoid viscous fingering.

The best column type is therefore very dependent on the requirements of the laboratory.

Q. Do you expect polymeric columns to be stable and tolerant to switching eluents? Or do you like to have a column set devoted to a particular solvent and then switch columns when switching solvents? What if you repeatedly change between pure tetrahydrofuran (THF) and THF with small amounts of additives, such as acids or amines?

A: In general, with solvents that are of a similar polarity to the packing material — such as THF, chloroform, dichloromethane, or toluene for styrene-divinylbenzene columns — exchanging the solvents should not harm the columns. However, it is advisable to exchange solvents slowly at reduced flow-rates of 0.3–0.5 mL/min. The solvent leaving the column should go directly to the waste and the detectors should be disconnected. There is no reason in principle not to exchange solvents; however, time might be an issue because completely re-establishing swelling equilibrium after going from one solvent to another often takes longer than reaching a stable RI-baseline.

For solvents that differ substantially in solvent polarity from the column material, we recommend using different columns because of the different swelling of the gel. We even recommend ordering such columns in the solvent of use. Please note that in many of these cases a different stationary phase polarity might be the better choice to avoid interactions.[4]

For the exchange between pure solvent and solvent with additives (amines, acids, salts) we do not see any problems with switching solvents back and forth.

In any case, columns should be stored in pure solvents without additives (no salts, amines, acids). The exception is columns for aqueous applications where a small amount of methanol or NaN_3 (0.05 g/L) should be added to avoid growth of algae.

Q. Does changing solvent composition by addition of salts or other co-solvents require recalibration of the detectors? Should the standards be run with the same modifier as you use for your sample?

A: Calibration is always an issue in GPC/SEC. There are two types of calibrations that can be applied:

1. Nearly all users perform a column calibration where they measure the elution volumes of calibration standards with different molar masses and plot the logarithm of the molar mass (or size) against the elution volume to construct a calibration curve (see also question above).
2. In some cases, users need to also calibrate their detectors in a high performance liquid chromatography (HPLC) type of detector calibration. This is often required when doing multi-detection GPC/SEC (triple-detection, light-scattering, or viscometry) or when they want to determine concentrations or perform copolymer analysis. In these cases different concentrations are measured to determine the detector responses.

In both cases we always recommend to apply the same conditions for calibration as for operation. So the standards should be run with the same modifiers or with the same co-solvents, and standards and samples should be prepared from the solvent bottle that also supplies the pump.

Q. Do you find some salts more/less corrosive to the instrument than others? For example, dimethylformamide (DMF) with LiBr is hard on the instrument, but gives good results.

A: Halides are usually more corrosive than other salts, and chlorides are more aggressive than bromides. Unfortunately, LiCl and LiBr have better solubilities than other lithium salts in commonly applied organic solvents; in addition, because lithium is more superior in breaking down aggregates than other counter ions, there are not too many options.

In aqueous solvents, neutral salts like nitrates or sulphates can be applied instead of NaCl to reduce the danger of corrosion. The main reason for salt addition is to shield electrostatic interaction and reduce the breakdown of aggregates.

An important factor when using salt solutions is to ensure that the system always runs with fresh solutions and that it is not left to stand for a long time. Always apply a low flow-rate to avoid salt crystallizing out and heavy corrosion. If the system is not in use for a long period of time it should be transferred into pure solvent before switching it off.

Summary

As with other analytical techniques, small details can have a big impact in GPC/SEC. Selecting the best column option and performing proper method development is definitely time-consuming, but can save much more time afterwards. A stable, robust method is much cheaper in the long run than choosing the wrong settings in the beginning. It is therefore important to always ask questions and so we encourage all readers to share their questions with us because there are a lot more answers that can help users make educated choices.

References

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