GPC/SEC eBook Series

GPC/SEC Columns

What you should know when you need to analyze polymers, biopolymers, and proteins

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About this eBook series

GPC/SEC *Tips & Tricks* articles have been published in more than 60 editions of LC/GC’s digital magazine *The Column* over the course of 10 years. These *Tips & Tricks* are designed to support GPC/SEC users in their daily work, providing comprehensive overviews on different aspects of this powerful technique.

This eBook series was created to have all published topics at a glance.

The topics of these eBooks will cover:

- GPC/SEC theory and background
- GPC/SEC columns
- GPC/SEC detection
- GPC/SEC troubleshooting
- GPC/SEC applications

Each eBook contains five to eight different *Tips & Tricks* publications that have been updated with the latest information, new examples, and figures.

To allow new users to GPC/SEC a continued reading experience, content has been edited, resulting in some differences compared to the original publications.

Nevertheless, the original spirit is maintained. So, the publications are independent references that allow users to read only the dedicated publication of interest.

Introduction to GPC/SEC columns

The choice of a column (stationary phase and surface chemistry) is the heart of any LC system, and the key to good results. The same criteria apply to the selection of GPC/SEC columns. There is a large variety of GPC/SEC columns available on the market. They come in different dimensions (as preparative, analytical, and microbore columns) and are packed with different types of stationary phases. Stationary phases are made of different-sized particles with a variety of different-sized pores.

Finding the perfect column(s) to obtain long-term reproducible results can be quite challenging. Choosing one of the different preconfigured and thoroughly tested column sets with particle size and pore size well-matched to a particular molar mass range simplifies the task.

Resolution, separation range, solvent consumption, and analysis time are key factors that must be balanced in every lab.

Depending on application and requirements, such as with routine QC, R&D, or when dealing with a variety of different samples, one of these parameters can overpower others during column selection.

It is important for scientists to understand the basic principles to make well-suited choices.

The first two sections of this eBook are dedicated to understanding the variables that influence the choice of stationary phase material, particle size, and porosity.

The third section discusses pitfalls that the scientists must avoid when selecting the final column or column combination.

The last sections of this eBook provide some practical tips for column installation and testing, as well as providing links to other resources to help you manage column selection.

1.1. How to determine the correct stationary phase for GPC/SEC

Driving forces in a GPC/SEC separation

Gel permeation chromatography/size exclusion chromatography (GPC/SEC) is a well-established technique for the analysis of all soluble macromolecules (synthetic and biological) for the determination of molar mass, molar mass distribution, chemical composition, and molecular architecture.

GPC/SEC is a liquid chromatography (LC)-derived separation technique that uses the same standard LC instrumentation as other LC packed-column techniques, such as reversed-phase LC (HPLC/RPLC), interaction polymer chromatography (IPC), and ion-exchange chromatography (IEX). The key differences between the various LC techniques are the type of stationary/separation phase used in the packed columns and the mobile phase used to dissolve the sample of interest.

Any chromatographic separation process can be described by the distribution coefficient K, defined as the ratio of the sample concentration between stationary (a_s) and mobile phase (a_m):

$$K = \frac{a_s}{a_m} = exp\left[-\frac{\Delta G}{RT}\right] = exp\left[-\frac{\Delta H - T\Delta S}{RT}\right]$$

where:
ΔG = Change in Gibbs energy

T = Absolute temperature

ΔH = Change in enthalpy

ΔS = Change in entropy

R = Universal gas constant 8.314 J/K mol

This equation can be divided into two parts:

- An enthalpic-driven process, enabling interactions (HPLC/IPC)
- An entropic-driven process, enabling an interaction-free process

Stationary phases for GPC/SEC ideally allow an entropic-only separation, according to the size or hydrodynamic volume of macromolecules in a sample.

$$K_{IPC} = exp\left[-\frac{\Delta H}{RT}\right] \text{ and } K_{SEC} = exp\left[\frac{\Delta S}{R}\right]$$

with $0 < K_{SEC} < 1$ and $\Delta H = 0$ and $K_{IPC} > 1$ and $\Delta S \approx 0$

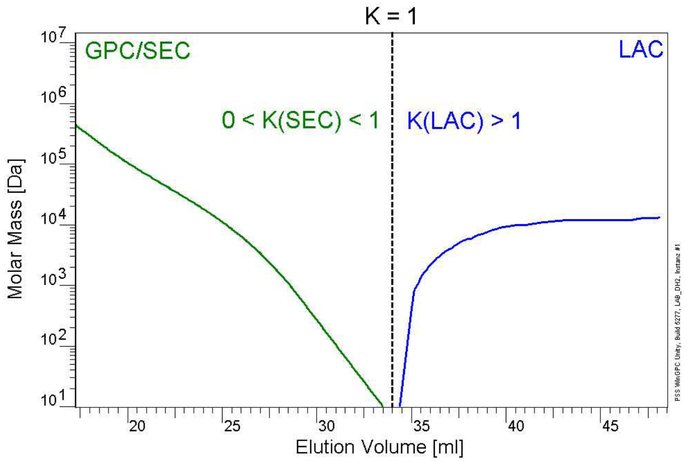

Figure 1. Molar mass versus elution volume plot, demonstrating chromatographic behavior in GPC/SEC and IPC.

Interactions between the sample and stationary phases must be eliminated or suppressed for optimal sample separation in GPC/SEC mode. This behavior is also required for advanced characterization with mass-sensitive detectors, such as online viscometers.

Interactions can be either attractive or repulsive. If the affinity of the sample to the stationary phase is greater than the affinity to remain within the mobile phase, the resulting interaction is attractive and leads to adsorption. This kind of interaction is successfully applied in IPC to separate copolymers according to chemical composition.[1]

Repulsive interaction between sample and stationary phase results in the sample not penetrating the actual pore. The sample only partially migrates into the pore. A strong polyelectrolyte sample, such as polystyrene sulfonate, elutes much earlier from a negatively-charged stationary phase than a neutral sample, such as pullulan (a polysaccharide), of comparable size. A separation according to size is still possible, but universal calibration will fail because of differing interactions of the samples.

Influences on method development

The goal of method development in GPC/SEC is balancing potential interactive effects between the sample, mobile phase, and stationary phase to eliminate any interactions.

Often, sample and solvent polarities are similar for a given analytical application, so a stationary phase with corresponding similar polarity must be selected. Only when a “net zero polarity” condition has been achieved can there be confidence that a robust GPC/SEC method with high reproducibility and a guarantee of long-term stability has been developed.

For column packing material selection, in which polarities of three parameters are reasonably well known, an equilateral triangle can be used as visual guide, where each side of the Magic Triangle represents one parameter: sample, mobile phase, or stationary phase polarity. Figure 2 shows the Magic Triangle for all types of mobile phases.

Table 1 shows examples of sample, mobile phase, and stationary phase combinations, organized by polarity. Moving equal-sided triangles up and down the polarity axis in the Magic Triangle can help identify the best choice for a mobile phase-stationary phase pair for each sample.[2] For a given water-soluble sample (e.g., polyacrylic acid), a hydrophilic, polar stationary phase matrix should be selected.

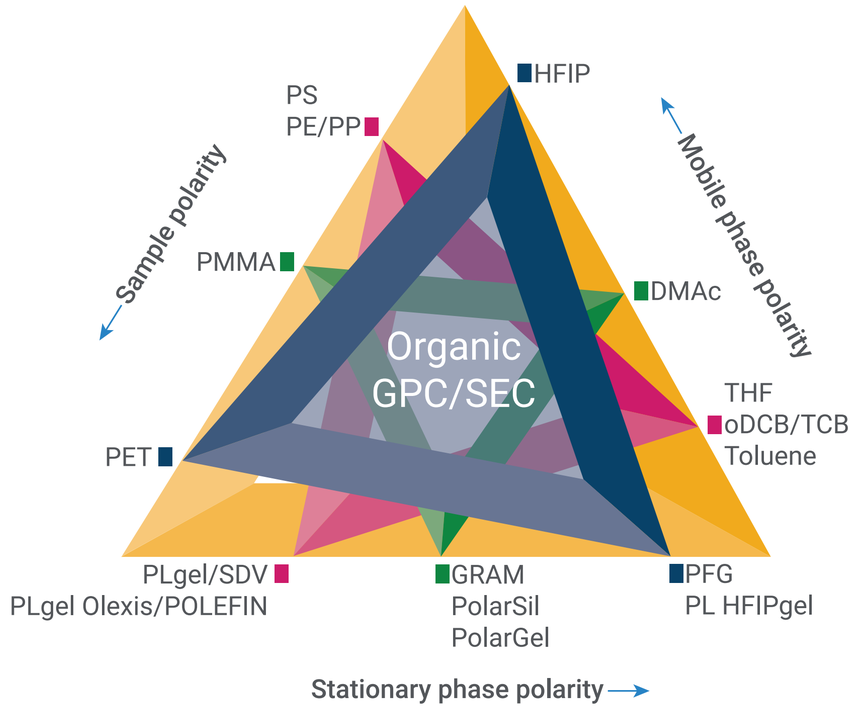

Figure 2. The Magic Triangle, balancing the polarities of the components.

Table 1. Balanced polarity axis of different chromatographic systems.

	Polarity	Sample	Mobile Phase	Stationary Phase
▲	Low or nonpolar	Polystyrene	Toluene	SDV/PLgel
▲	Medium	Polymethyl methacrylate	Dimethylacetamide (DMAc)	GRAM, PolarGel
▲	Medium to high	Polyethyleneterephthalate	Hexafluoroisopropanol	PFG, PL HFIPgel

The Magic Triangle concept can be extrapolated to describe a range of column stationary materials for neutral, cationic, and anionic samples in aqueous applications.

Charged samples must be shifted to a fully dissociated state (salt form) in aqueous GPC/SEC, to suppress any kind of hydrophobic interaction. Hence, water-based mobile phases must be pH-adjusted (Figure 3). Polyacrylic acid, as a weak, organic acid, is fully dissociated at pH 9. No hydrophobic interaction (i.e., hydrogen bonding) occurs. It can be analyzed on hydroxylated methacrylate-based material (SUPREMA). Strong polyanions, such as polystyrene sulfonates, must be analyzed at a high pH.

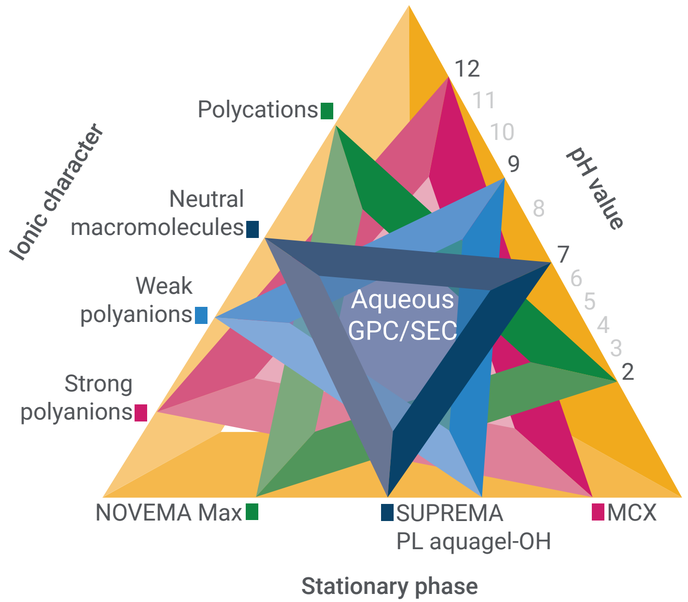

Figure 3. The Magic Triangle for aqueous applications.

Requirements for reliable GPC/SEC measurements

Balanced polarity is a minimum prerequisite for samples with many functional or ionic groups (e.g., polyelectrolytes) but is not sufficient for interaction-free GPC/SEC.

To suppress ion-exclusion, the addition of low-molecular, neutral salts, or use of buffer solutions is necessary. These salts can minimize interactions with the stationary phase and suppress sample aggregation. However, the salt concentration should be selected carefully, as there is only a small concentration range where hydrophilic and hydrophobic interactions can be avoided.[3]

Figure 4 depicts elution volume dependence of a sample as a function of increasing ionic strength. Too low of an ionic strength yields an increased elution volume, due to adsorption or ion exclusion. Increasing the ionic strength to 0.5 to 1.5 mol/L ensures essential GPC/SEC separation. Above an ionic strength of 1.5 mol/L, hydrophobic interactions are dominant, and the elution volume increases again.

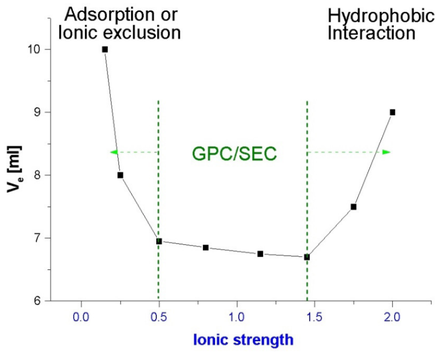

Figure 4. Salt concentration/ionic strength range yielding true GPC/SEC separation.

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2. Reinhold, G. Lecture: Practical Aspects of Stationary Phases Used for SEC, 7th Dresden Polymer Discussion, **1999**.
3. Reinhold, G. The Influence of the Stationary Phase Polarity on GPC/SEC Separations, *Austrian Polymer Days* **2005**.

Originally published in *The Column*, December **2007** by authors Thorsten Hofe and Guenter Reinhold.

1.2. Factors affecting resolution and separation

Once the suitable stationary phase has been identified, column selection in GPC/SEC is then about finding the optimum balance between separation range, analysis time, solvent consumption, and resolution:

- A wide separation range is required for highest versatility and applicability, whereas good resolution is necessary for comprehensive analysis, especially for detailed molar mass distribution (MMD).
- Analysis time and solvent consumption are important factors for quality control, high throughput GPC/SEC, and when using expensive solvents.
- Resolution is dependent on a range of factors, including column material, particle size and packing quality, pore size and pore size distribution, solvent viscosity, and flow rate, as well as sample concentration and mass transfer considerations.

A measure of separation is the specific resolution, R_{sp} (recommended for GPC/SEC).[1] For specific resolution, peak standard deviation being proportional to peak width, and slope of the calibration curve are required (see section 1.5).

Linear columns versus single pore size columns

Two different column formats are available on the market:

- Single porosity columns with narrow pore size distribution (monomodal)
- Linear or mixed-bed columns with broad or multimodal pore size distribution

For any column format, the separation range capacity itself is limited by available pore size distribution. Calibration curves help to visualize the different resolution areas.

For single porosity columns, separation range is concentrated in a narrow molar mass range, yielding calibration curves with very flat or shallow slopes in this particular region. Hence, single porosity columns have a limited separation range, but offer high resolution. In contrast, columns with broad pore size distributions provide a larger separation range, meaning that the calibration curve has a steeper slope, and resolution is decreased.

Figure 1 shows that for a given elution volume range ΔV ($V_2 - V_1$), a linear column spans a wider molar mass range than a comparable single porosity column. However, the latter column clearly exhibits higher resolution, and thus, better separation over the covered molar mass range.

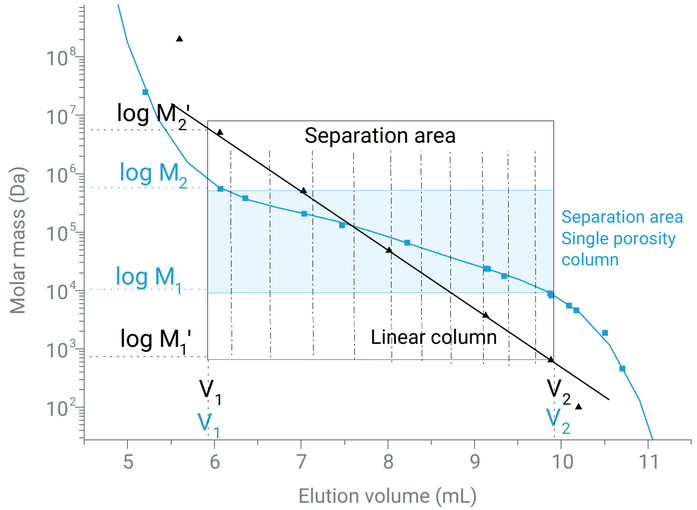


Figure 1. Comparison of the separation range of a linear column and single porosity column.

The effect of porosity on separation range

The porosity of a column is directly related to the separation range of the column. Figure 2 compares the molar mass range of single pore size columns with linear columns/mixed-bed columns (which contain particles of different porosities measured in Å).

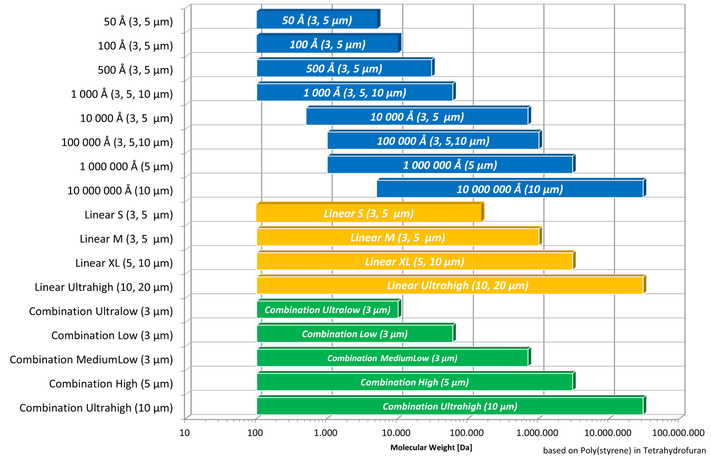


Figure 2. Molar mass range comparison for single pore size and linear columns.

Increasing resolution and separation range

To increase resolution and/or separation range, a very simple approach is used:

Instead of using just one column, multiple columns are combined into a set (or column bank) to generate more pore volume or increase the resolution. Two to four columns in a set (including precolumn) are common in GPC/SEC.

- A suitable combination of different-sized single-porosity columns will effectively extend the separation range, while maintaining a high resolution level. Polymers are baseline-separated over several molar mass decades.
- A combination of linear columns (or single pore size columns of the same type) will improve the resolution, but not the separation range. The combination of two columns with the same porosity (single porosity or linear/mixed-bed/multipore) flattens the calibration curve, and therefore increases the resolution. As a guideline, combining two columns of the same porosity improves the resolution (R_s) by a factor of 1.4.

Analysis time, eluent consumption, and pressure increase disadvantage the column set concept. Increased pressure might entail flow rate reduction and/or temperature increase for more favorable chromatographic conditions, especially for high molar mass polymers. There is also the potential danger of porosity mismatch for all column types.[2]

Figure 3 depicts the difference in performance between a linear column, and a combination of three single-porosity columns. This column combination demonstrates a noticeably better resolution across the entire molar mass range (i.e., increased elution volume range) with a larger separation range. A polymer mixture consisting of four different molar masses (PEO ReadyCal Black) is well baseline-separated. Alternatively, the analysis time of one linear column is three times faster than a combined column set, which might be better for product screening.

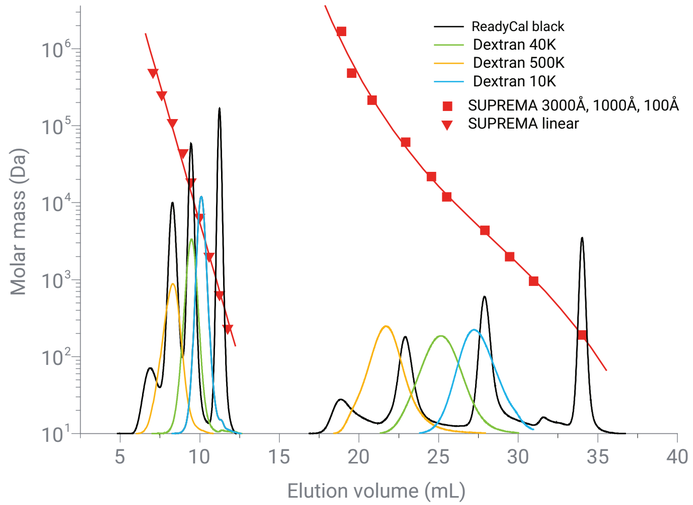


Figure 3. Comparison of a linear column with a column combination of three single-porosity columns.

How do chromatograms look on different column sets?

Column sets are often optimized for different separation ranges (compare Figure 2 where the combinations match the ranges of linear columns). There are sets for high molar masses and for low molar masses/oligomers. Figure 4 depicts a comparison of a sample mixture injected onto two different column sets.

Other parameters influencing resolution

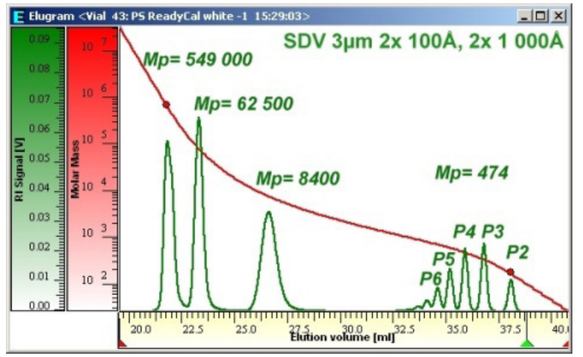
Generally, all parameters improving mass transfer lead to better resolution. Parameters that can be directly influenced by GPC/SEC users are:

A) Particle size

Plate count and column permeability decrease with increasing particle diameter. Small particle size columns offer better resolution. Figure 5 displays a comparison of a PS oligomer mixture analyzed on the same column material, with the same pore size range, and different particle sizes. Mass transfer of the 3 µm material is much better, and results in increased resolution. Therefore, if size and rigidity of the polymers permit, and no shear degradation occurs, higher-end small particle columns would be a good investment for higher resolution.

As a general rule, oligomers to medium molar mass (i.e., 500 KDa) samples in less viscous solvents and proteins tolerate particle sizes around 3 µm. Particles in sizes of 5 to 10 µm are used for higher molar masses or more viscous solvents (such as xylene). For high molar masses and highly viscous solvents, 10 to 20 µm particles sizes are used.

A



B

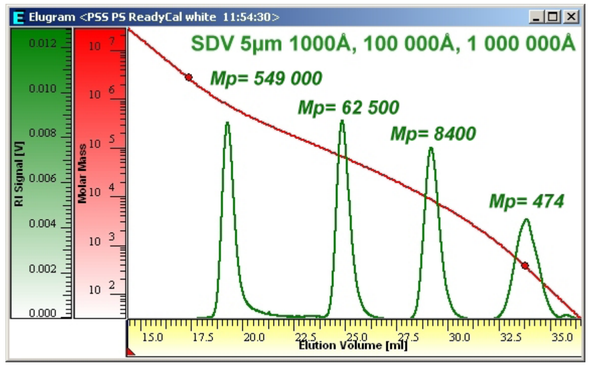


Figure 4. (A) Mixture of four different polystyrene standards analyzed on a column set optimized for high separation capacity in the oligomeric region. The set can resolve several oligomers, baseline-separated. (B) The same mixture analyzed on a column set optimized for medium and high molar masses. Resolution in the oligomeric region is less, but this set separates medium and higher molar masses more sufficiently than the set in (A).

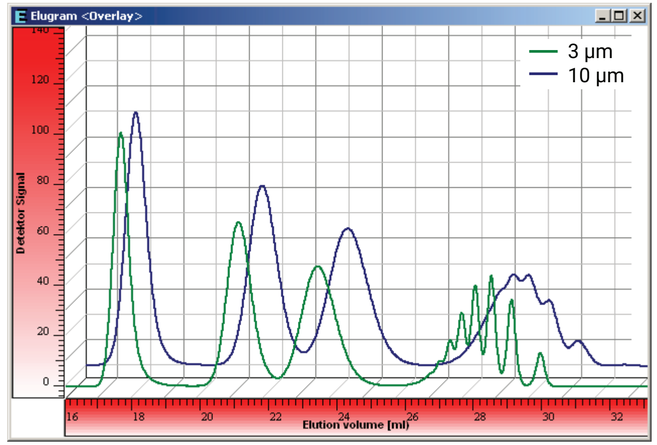


Figure 5. Comparison of a PS oligomer separation on a 3 µm and 10 µm particle size column set.

B) Flow rate

A flow rate of 1 mL/min is generally used with analytical GPC/SEC columns. An inner diameter of 7 or 8 mm is the best compromise between resolution and analysis time. Especially for higher molar masses, lower flow rates might be required. For oligomers, a flow rate decrease generates increased resolution.

C) Temperature

A temperature increase will usually also result in improved resolution due to enhanced mass transfer. However, this is not applicable for all polymers. For example, better resolution for polyethylene glycol (PEG) in aqueous solutions is achieved at lower temperatures.

Figure 6 illustrates several effects, displaying how optimization of particle size, number of columns, flow rate, and temperature can substantially increase resolution. In Figure 6, a dextran was analyzed using different columns and conditions. The best resolution was obtained with a low flow rate on two 5 µm columns.

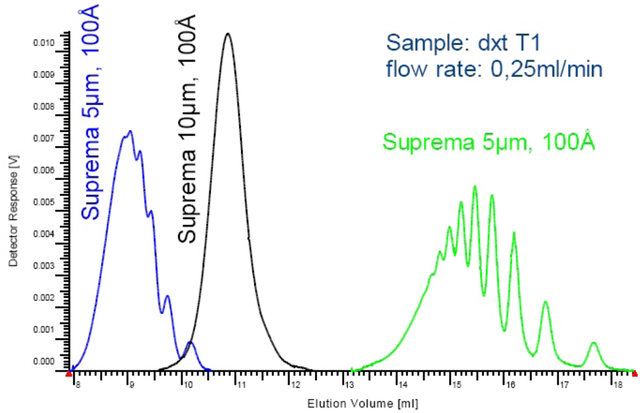


Figure 6. Optimizing particle size, flow rate, and temperature.

Practical advice for column selection and resolution optimization:

- Avoid interactions by selecting the matching stationary phase polarity.
- Use the lowest particle size possible. For oligomers or proteins, small particle sizes can be used without limitations.
- Combining columns of the same porosity will improve resolution. Adding columns with other pore sizes will increase separation range.
- When buying columns, ask the manufacturers for mismatch-free combination examples. Do not combine linear/mixed-bed/multipore columns with single porosity columns to boost resolution in the low molar mass regime. Do not confuse peak shoulders resulting from mismatch with high resolution.
- If analysis time is not an issue, try lower flow rates. This will not impact solvent consumption.

References

1. Reinhold, G. How to Expand the Life Time of Columns, LC/GC. *The Column* **2009**, *12(7)*.
2. Hofe, T. Beware of Mismatch, LC/GC. *The Column* **2008**, *4(20)*.

Originally published in *The Column*, February **2011**, by author Daniela Held.

1.3. Molar mass separation range: Dos and don’ts for selecting columns

The concept of specific resolution exists as a way for GPC/SEC users to understand and compare resolution, and to determine the influence of its effect on molar mass accuracy.[1] Review of the slope of a calibration curve gives a good visual guide for resolution. The flatter the slope, the higher the resolution.[2]

Separation on one column with good resolution, but with limited separation range, suffices for protein, antibody, or oligomer applications. However, efficient separation over a large range is required for broad distribution synthetic or natural polymers. For this reason, columns are often used in series. This approach is very successful, but has some pitfalls such as mismatch, yielding artifacts in chromatograms and molar mass distributions.

Selecting the upper molar mass separation limit

Often, GPC/SEC users only know the approximate weight-average molar mass (M_w) when having to select a column or a column set. Inadequate choices can be made if the width of MMD (i.e., the ratio of M_w/M_n, referred to as the Dispersity (Đ)) of the sample is neglected.

If the Đ of a given sample is approximately 2 (a typical value of synthetic polymers produced by free-radical polymerization), the expected molar mass range is very broad. As a general rule, users should select an upper molar mass limit 10 times higher than the anticipated M_w of the largest sample to be analyzed.

Figure 1 depicts the molar mass distribution of polystyrene with M_w = 181,200 Da and Đ = 2.26 (European reference material). Although M_w of this polymer is fairly low, the sample comprises molar mass fractions up to 2 × 10^6 Da.

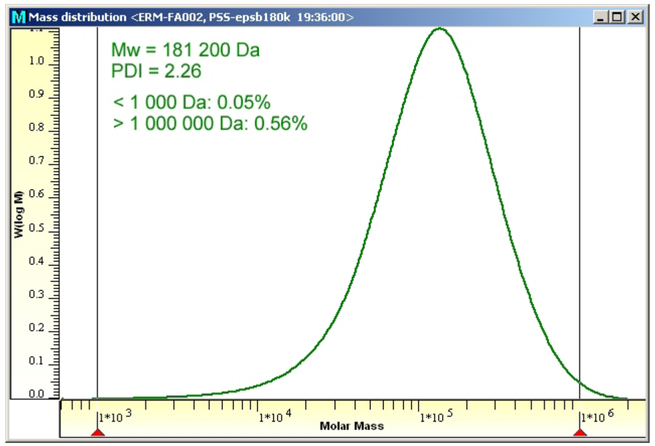


Figure 1. Molar mass range of a PS with a Đ of approximately 2 and a medium M_w.

Figure 2 displays an example of a sample that encompasses molar mass fractions exceeding the exclusion limit, i.e. the upper molar mass separation limit. It shows the refractive index signal (RI, green trace) overlaid with the respective calibration curve (red trace). The two red dots denote the highest and lowest molar mass of the calibration standards. Exceeding the exclusion limit will generate an artificial shoulder in the chromatogram at approximately 6 mL elution volume. This shoulder is not sample related, but induced by resolution change, indicated by slope change in the calibration curve. This problem can be resolved by adding a second, correctly matched column with a higher porosity (and therefore, a higher exclusion limit).

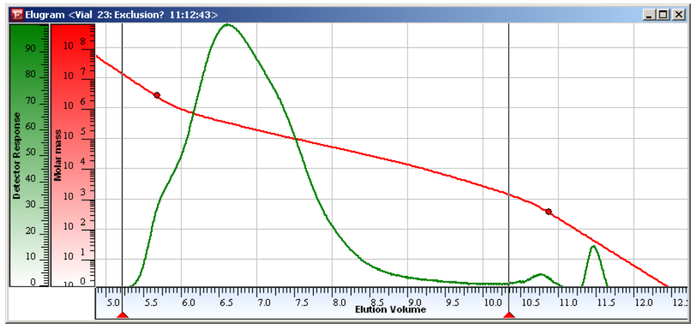


Figure 2. Selecting too low of an upper molar mass separation limit can yield artificial shoulders in chromatograms.

Selecting the right column combination

Unfortunately, it is not possible to combine linear/mixed-bed columns and/or single porosity columns in every combination. Figure 3 illustrates an example of four different dextran samples, analyzed on a set of two analytical GPC/SEC columns with widely different porosities. One of the samples, dextran 40 (black trace) seems to be bimodal, as it exhibits a shoulder. However, this shoulder is not sample related, and does not show in a single column run or on a different column set (compare Figure 4).

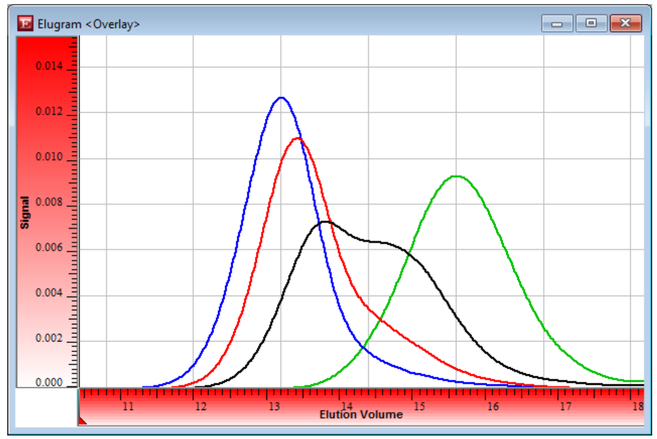


Figure 3. Is the dextran bimodal?

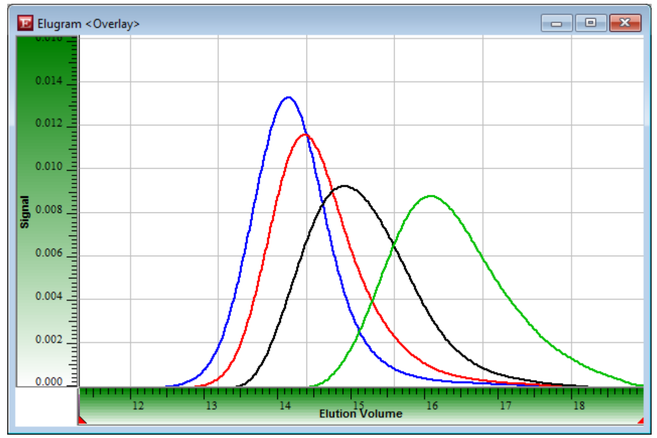


Figure 4. Dextrans on a mismatch-free set.

Subsequent processing of the raw data from Figure 3 erroneously yields a shoulder in the molar mass distribution. Problematically, small changes in the manufacturer’s synthesis recipes can also cause this shoulder to shift or disappear. Based on this notion, reproducible results are hard to achieve in the long run.

Porosity mismatch is not always readily visible in a calibration curve. Figure 5 depicts a calibration curve example of a very small and a very large porosity column, in series. The resulting calibration curve exhibits two regions with different slopes, as well as an inflection point. Such a combination would generate chromatographic artifacts.

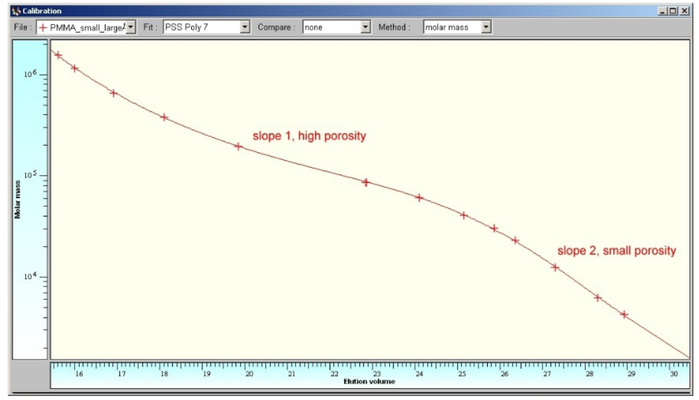


Figure 5. Calibration curve of two columns with nonmatching porosities: the curve has two different slopes resulting in erroneous chromatograms with shoulders.

In contrast, the calibration curve could look normal, but there could still be an invisible mismatch problem. This mismatch can only be detected by probing the region of the inflection point using broad distribution reference materials.[3]

It is not possible to eliminate this problem with advanced calibration data fitting, even if absolute detection with online light scattering is used. Optimizing separation by combining correctly matched columns is the only solution.

Conclusion

There are some general rules that hold essentially true for all columns, independent of manufacturer. Applying these three basic rules will help establish long-lasting GPC/SEC methods that can be applied with high reproducibility.

A) Ensure that the upper molar mass separation limit is sufficient by taking the sample Đ into account. Verify that all sample fractions elute in a region with sufficient separation by superimposing the calibration curve over the raw data of the sample.

B) Avoid combining:

- Linear columns of different types
- Linear columns with single porosity columns (e.g., linear + 100 Å)
- Single porosity columns of different particle sizes (or only do so following the manufacturer’s recommendation or by conducting careful mismatch tests)

C) Investigate unexpected shoulders carefully. Review the calibration curve regarding slope changes and use broad distribution reference materials to detect hidden porosity mismatch. Porosity mismatch can even occur within a single column, if the manufacturer incorrectly blends packing materials to extend molar mass range in a linear/mixed column. Also, do not assume that a linear/mixed-bed column that has a linear calibration curve with a specific slope (in, e.g., THF) will have the same linearity and slope in, for example, toluene.

References

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Originally published in *The Column*, December **2013**, by author Daniela Held.

1.4. How to install GPC/SEC columns

For proper column installation, it is important to be familiar with column characteristics such as solvent compatibility, maximum flow rate, or maximum operating temperature.

Installation of a GPC/SEC column is similar to installing a HPLC column. However, a few important differences should be considered.

Similar to HPLC stationary phases, GPC/SEC columns can be grouped into silica-based and polymer-based. Silica-based stationary phases have the advantage of higher pressure stability and solvent compatibility. They often provide excellent resolution in a narrow molar mass range. Alternatively, polymer-based materials provide broader separation ranges. These are often easier to combine into column sets (banks), increasing the separation range. These are less susceptible to interaction with samples.

For both column types, it is important to never exceed the maximum column pressure during installation and to ensure that air introduction is avoided.

Consider before installation

A) Solvent compatibility

Verify that the solvent to be used is compatible with the column(s).

If the mobile phase of the GPC/SEC system and solvent in the column are different, change the mobile phase on the system to match that of the column.

Use intermediate solvents if the mobile phase of system and column are immiscible.

Follow the procedure for column installation. Following installation, the columns can be switched to a required solvent by following the instructions in the column user manual.

B) Tubing

Tubing length between columns and detectors should be minimized to avoid dead volume that results in band broadening.

Additionally, the inner diameter (ID) of the tubing must be adapted to the column ID and application. This is especially important when narrow bore or preparative columns are used. Narrow bore columns require small ID tubing. Preparative applications that are operated at high flow rates often require larger IDs.

C) Fittings and ferrules

It is good practice to replace existing fittings and ferrules, especially when columns from a different manufacturer are installed.

Figure 1 displays tubing with fitting and ferrule. The stop depth, x, varies for different manufacturers and sometimes brands. When trying to install columns from a different manufacturer with existing fittings, x can either be shorter (resulting in dead volume and poor separation) or longer (with the danger of destroying the column frit when tightening the fitting).

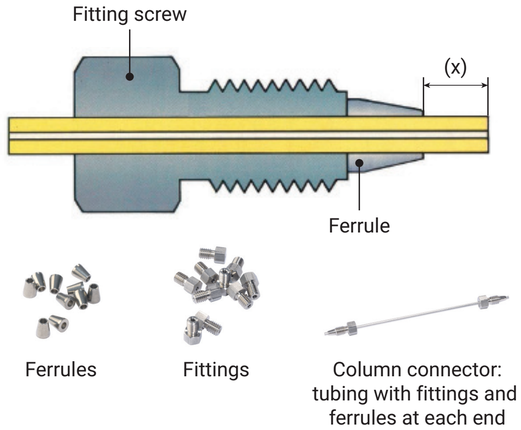

Figure 1. Stop depth (x) varies for different manufacturers. Replace existing fittings and ferrules when changing the columns.

Column installation

1. Set a low flow rate (0.2 mL/min) to avoid air introduction into the system.
2. Check if solvent is dripping out of the tubing.
3. Remove the plugs of the precolumn to be installed, and connect the column to the tubing in the correct flow direction (indicated by an arrow for most columns).
4. Insert the tubing until it bottoms firmly. Do not over-tighten the fitting. It only needs to be hand-tight, enough to seal.
5. Check if solvent is coming out of the other end of the column. If this is not the case, check if the fitting is too tight, or if air is leaving the column. An easy test to monitor air bubbles is to put the column end in a beaker with mobile phase.
6. In case of air trapped at the inlet: Install column in the reverse direction and fill the column with solvent, using a flow rate of 0.1 m/min until no more bubbles appear at the column outlet. Then change the column over to the correct flow direction. Use a flow rate of 0.1 mL/min until no more bubbles appear.
7. Connect the next column only after some eluent has dripped from the column outlet.
8. Flush columns with at least 3 to 5 column volumes before reconnecting the detectors.
9. Increase flow rate slowly to operating flow rate in 0.2 mL/min increments every 5 minutes. Once the system pressure has stabilized, check for leaks at the new connections.

Column order in a column set

In general, the order of columns in a column set will not influence the results of GPC/SEC analysis.

If not recommended differently by the manufacturer, it is recommended to install the columns with decreasing porosity if a column set consists of different porosities. This will result in better separation since the viscosity of the injection band is dissipated faster. High molar mass compounds are the biggest contributor to viscosity. If the column separates large molecules, the viscosity will decrease faster, facilitating the diffusion process into

pores.

An exception can be made if columns with a low pressure stability are used. If so, it is better to install these columns at the end of a set to protect it from potential pressure pulses (e.g., from a defective pump).

Check column and system performance

Remember that GPC/SEC columns need a certain time to equilibrate. As a rule, a minimum of five column volumes (ideally 10, when exchanging solvents) are required until the columns are equilibrated.

Before calibrating new columns, it is suggested to check column performance by, for example, measuring the plate count. It is recommended to follow the manufacturer’s test procedure. Keep in mind that the plate count depends on many parameters that are not necessarily column related. Large dead volume in the system, such as wrong tubing, fittings, or ferrules can result in low plate counts.

If the plate count test for a column set fails, it is advised to test the columns separately to identify the defective column (or precolumn). After a successful plate count test, the columns should be calibrated, and resolution should be determined. Following this test, the system is ready for samples analysis.

Conclusion

- When installing GPC/SEC columns, it is always recommended to have a small flow, to avoid air being trapped in the column.
- Although modern GPC/SEC columns are much more stable than columns of the past, it is good practice to install the columns in the recommended flow direction.
- The sequence of columns in a column set does not affect results, but may influence column lifetime and resolution. If not recommended differently by the manufacturer, the general recommendation is installation with decreasing porosity.

Originally published in *The Column*, February **2014**, by author Daniela Held.

1.5. How to test GPC/SEC columns

Column plate count, asymmetry, and resolution

Performance of columns is often described by the plate count N (or plate count/m), peak symmetry or asymmetry, and the specific resolution (R_{sp}). There are several national and international standards that provide acceptance criteria for the various parameters in specific solvents. These include ISO 13885, DIN 55672, and ASTM D 5296-05, among others. It is good practice to monitor these parameters on a regular basis.

Before selling a column, the manufacturer tests a column for performance, which is documented in a Certificate of Analysis, including conditions. After installing the columns, it is recommended to repeat this test in the user’s lab using the same conditions.

Please note that comparisons of overall column performance can only be done if pertinent parameters are maintained. Mobile phase, test material, flow rate, temperature, column loading, detector, and system (including tubing) should match. Otherwise, the numerical results are not comparable.

To determine plate count and asymmetry, a monodisperse sample can be injected. Detailed experimental conditions can be either found in a corresponding GPC/SEC standard, or on the column quality certificate.

Additional reference materials for a variety of common GPC/SEC solvents are reported in Table 1. An injection volume of 20 µL (or less) for these materials is sufficient, whereas the concentration should be adapted to the amount of columns used (one column: 0.2 g/L, two columns: 0.5 g/L, three columns: 1 g/L).

Table 1. Suitable reference materials for plate count, peak symmetry, and resolution determination.

Mobile Phase (Sorted by Polarity)	Plate Count	Resolution	Recommended Operating Temperature (°C)
Toluene	BHT	Polystyrene	30 to 40
Tetrahydrofuran	BHT	Polystyrene	30 to 40
Chloroform	BHT	Polystyrene	30 to 40
1,2,4-Trichlorobenzene	BHT	Polystyrene	140 to 160
Dimethylacetamide	Methyl isobutyrate, e.g., mm102	Polymethyl methacrylate	60 to 80
N-Methylpyrrolidone	Methyl isobutyrate	Polymethyl methacrylate	60 to 80
Dimethyl sulfoxide	Methyl isobutyrate	Polymethyl methacrylate, alternative: pullulan*	60 to 80
Dimethylformamide	Methyl isobutyrate	Polymethyl methacrylate	60 to 80
Hexafluoroisopropanol	Methyl isobutyrate	Polymethyl methacrylate	30 to 40
Water	Ethylene glycol	Pullulan	30 to 60

* Weak PMMA signals due to small dn/dc of PMMA in DMSO; if S/N ratio is not sufficient, use pullulan.

Calculation of the theoretical plate count per meter, N_{th} (1/m), uses peak position and peak width at half peak height $w_{1/2}$:

$$N_{th} = \left(\frac{V_p}{\sigma}\right)^2 = \frac{554}{L} \cdot \left(\frac{V_p}{w_{1/2}}\right)^2$$

with

σ = Variance estimated by the half-height method, $w_{1/2}$
V_p = Elution volume at peak maximum
L = Column length in cm

The asymmetry calculation depends strongly on the applied reference standard. DIN 56672 and ISO/EN 13885 define peak asymmetry as:

$$A = \frac{w_l}{w_r}$$

with W_l and W_r being peak widths on the left and right side of peak maximum (measured at 10% of the peak height).

ASTM defines asymmetry as $A' = W_r/W_l$, thus $A' = 1/A$.

Generally, resolution is more important, as it describes separation performance within a specific molar mass region. By injecting a mixture of polymer standards, resolution can be determined. Alternatively, a calibration curve can provide some required information, such as slope (D), if available.

The specific resolution R_{sp} reveals the resolution quality of two peaks, R_s, where molar mass differs by one order of magnitude:

$$R_{sp} = \frac{R_s}{lg\, M_1 / M_2} = \frac{0.579}{\sigma \cdot D}$$

Figure 1 illustrates the results of a test with butylated hydroxytoluene (BHT) in THF. Green numbers indicate that the acceptance criteria of the applied standard ISO 13885/DIN 55672 are met.

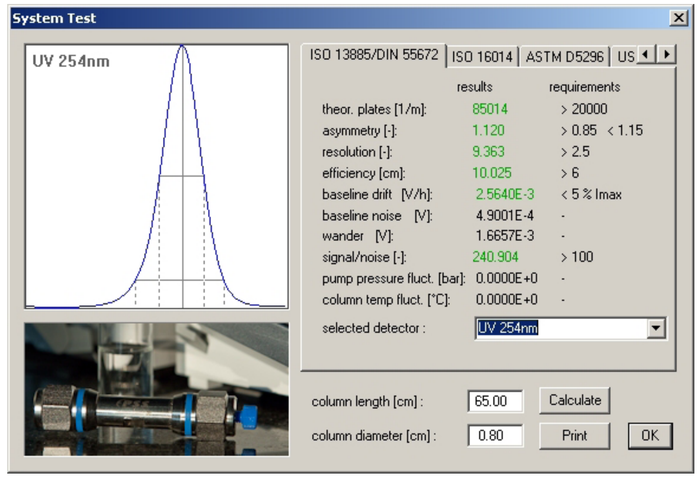

Figure 1. Example for plate count, peak symmetry, and resolution determination based on the acceptance criteria from ISO 13885/DIN 55672.

Regular plate count determination is strongly recommended, if many different sample chemistries or samples of unknown origin are analyzed. A test before and after analysis helps to identify decreasing column performance due to insufficient sample preparation, or to identify samples that cannot be analyzed under given conditions. In quality control settings, a control sample of the same chemistry as the main sample is run to ensure integrity of the method.

Mismatch tests

If a shoulder appears at a specific elution volume in a peak, a pore size mismatch could be a possible reason. Pore size mismatch can either occur in a column set, or in a single column with blended pore sizes.[1] In contrast to resolution tests, pore size mismatch tests require injections of broad distribution reference materials. These materials should be injected if unexpected shoulders occur in chromatograms.

Actions if the plate count test fails

If the plate count or asymmetry test of newly installed columns fails, it is recommended to inspect the column connections first. If the column manufacturer was changed, it is possible that the stop depth of the column heads is responsible for poor performance. Unfortunately, the stop depth varies for different manufacturers. Reusing existing tubing can often create a problem when new columns are installed.

If the stop depth is not the reason for test failure or poor performance, it is recommended to test columns individually. A test of the precolumn or guard column should also be conducted. A single defective column or precolumn within a set can result in an out-of-specification plate count or asymmetry. Defective columns need to be replaced. Should tests results be ambiguous, contact the column manufacturer for assistance.

References

1. Hofe, T. Beware of Mismatch, LC/GC. *The Column* **2008**, *4(20)*.

Originally published in *The Column*, August **2014**, by authors Friedhelm Gores and Daniela Held.

Glossary

Å	Ångström, unit of length equal to 10^{-10} m
BHT	Butylated hydroxytoluene
Da	Dalton (g/mol)
DMAc	Dimethylacetamide
Đ	Dispersity (Đ = M_w/M_n)
dn/dc	Refractive index increment
Eluent	Fluid used to elute a substance
Exclusion limit	Marks the upper limit of the separation capability of a column. Large analyte species can no longer penetrate the pores of the packing.
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
id	Inner diameter
LS	Light scattering
M_n	Number-average molar mass
M_w	Weight-average molar mass
Mobile phase	Liquid phase used on a chromatography system
MMD	Molar mass distribution
PEG	Polyethylene glyol
PMMA	Polymethyl methacrylate
PS	Polystyrene
RI	Refractive index (detection/detector)
R_{sp}	Specific resolution
SDV	PSS styrene-divinylbenzene
SEC	Size exclusion chromatography
S/N	Signal-to-noise ratio
Solvent	Liquid in which a solute is dissolved to create a solution
Stationary phase	Solid phase in a separation device on which materials will be separated
THF	Tetrahydrofuran

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Published in the USA, October 17, 2023
5994-5936EN

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