

Tips & Tricks: GPC/SEC

Increase Resolution and Separation Range

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GPC/SEC is used to separate, identify and characterize macromolecules with respect to their molar mass averages and molar mass distribution (MMD). The precision and accuracy of the results depends, amongst other parameters, on the selection of the proper separation columns.

A proper column selection in GPC/SEC always includes finding a compromise between separation range, analytical quality, analysis time and solvent consumption. A wide separation range is required for highest versatility and applicability, a good resolution is required for the in-depth analysis, especially for a detailed MMD. Analysis time and solvent consumption are important especially for quality control in high temperature GPC and when expensive solvents are used.

The dependence of GPC/SEC column characteristics and experimental parameters on the resolution is quite complex. Column material particle size and packing quality, pore

size and pore size distribution, solvent viscosity, temperature and flow-rate, as well as sample concentration and other factors influence the mass transfer and, therefore, the resolution.

A measure for the resolution can either be the plate count, N , or (recommended for GPC/SEC) the specific resolution, R_{sp} .¹ For the specific resolution the peak standard deviation (which is proportional to the peak width) and the slope of the calibration curve are required.

Column Concepts in GPC/SEC: Single Porosity vs Linear or Mixed-bed Columns and Column Banks

In general GPC/SEC columns can either be columns with a narrow pore size distribution (single porosity columns) or columns with a broad pore size distribution (linear, mixed-bed or multi-pore columns).

GPC/SEC separation capacity itself is limited by the available pore volume and depending on the slope of the calibration curve. For single

porosity columns the separation capacity is concentrated in a narrow molar mass range to yield a calibration curve with a flat or shallow slope in this region. Therefore, single porosity columns have a limited molar mass separation range but a high resolution in that range.

In contrast to that, a column with a broad pore size distribution provides a larger separation range, the calibration curve has a steeper slope and, therefore, less resolution.

To increase the resolution and/or the separation range a very simple approach is applied. Instead of just using one column, GPC/SEC users combine multiple columns to a column combination or a column bank. Two to four columns (plus a pre- or guard column) are typical in GPC/SEC.

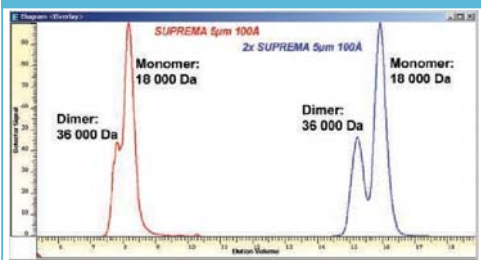
The idea behind a column combination is that more columns provide more available pore volume. If two columns with the



same pore sizes (single porosity or linear/mixed-bed/multi-pore) are combined, the calibration curve becomes flatter and the resolution increases by 1.4. Figure 1 shows the increased resolution for the separation of a protein monomer from the dimer, when the number of columns is increased.

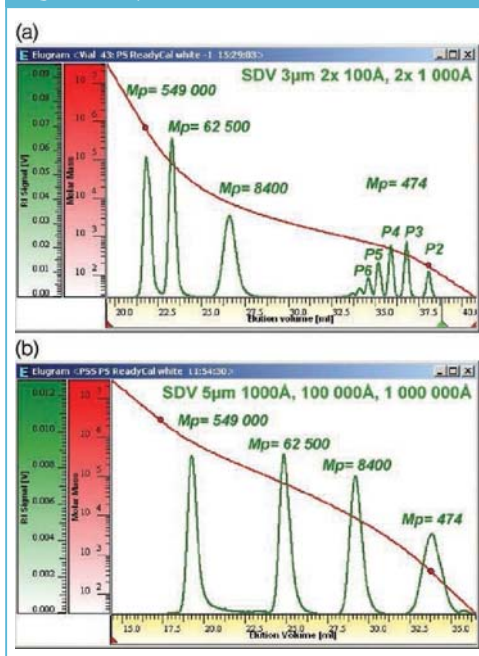
If columns with different porosities are combined the separation range increases. Figure 2 shows a comparison of the same sample mixture measured on two different column banks. In Figure 2(a) the columns are optimized for high separation capacity in the oligomeric region, Figure 2(b) is a typical example for a separation on a column combination optimized for the separation of medium molar mass macromolecules. This example also illustrates the influence of the slope of the calibration curve on the resolution

Figure 1: Separation of a protein monomer from the dimer on one SUPREMA 5 µm column and on two columns (porosity for both columns: 100Å). Flow-rate: 1 mL/min.



as well as the difficulty for inter-laboratory comparison of chromatograms. For comparisons between different labs it is always recommended to compare the molar mass distribution instead of the raw data.

Figure 2: (a) Mixture of four different polystyrene molar mass standards analysed on a column combination optimized for the separation of low molar mass oligomers. (b) The same mixture analysed on a column combination optimized for a wider separation range with less emphasis on oligomer separation.



Disadvantages of the column combination concept are that pressure, analysis time and eluent consumption increase. An increased pressure might result in the need to reduce the flow-rate and/or to increase the temperature to have better chromatographic conditions especially for high molar mass macromolecules.

In addition there is the potential danger of porosity mismatch for all column types.² Porosity mismatch often shows itself in peak shoulders that might be misinterpreted as better resolution, but are artefacts of a column/columns with mismatching porosities.

Other Parameters Influencing the Resolution

In general all parameters that improve the mass transfer lead to a better resolution. Parameters that can be directly influenced by GPC/SEC users are:

Particle size: Plate height and column permeability decrease with the particle diameter. Smaller particle size columns, therefore, provide a better resolution. Figure 3 shows a comparison of a protein mixture measured on the same column material with different particle sizes. The mass transfer for the 5 µm material is much better, resulting in an increased resolution. Therefore, if the molar mass and rigidity of the macromolecules permit and no shear degradation occurs, the higher prices for small particle columns are a good investment in higher resolution.

The general rule of thumb is that oligomers in low viscous solvents and proteins allow particle sizes around 3 µm. For medium molar masses 5–10 µm particles are used and for

Figure 3: Separation of a protein mixture on a SUPREMA column with 5 µm particle size compared with one of 10 µm particle size.

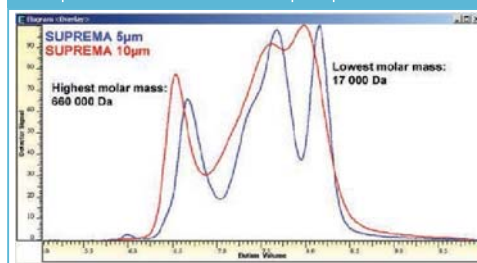
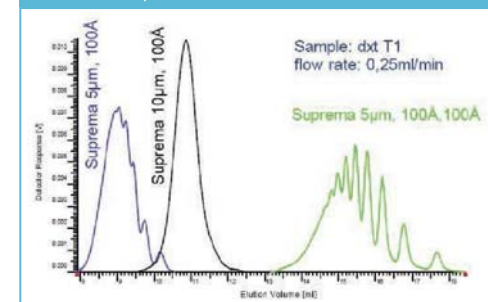


Figure 4: Analysis of a Dextran oligomer on one SUPREMA column 5 µm, on a SUPREMA 10 µm and on two SUPREMA columns 5 µm with reduced flow-rate.



high molar masses and high viscous solvents 10–20 μm particle sizes are applied.

Flow-rate: 1 mL/min flow-rate is often applied for analytical GPC/SEC columns with an inner diameter between 7 and 8 mm as the flow-rate with the best compromise between resolution and analysis time. A decrease of the flow-rate results in a higher resolution, particularly for higher molar masses. A flow-rate decrease can also result in an increased resolution for oligomers.

Temperature: A temperature increase will also normally result in a better resolution because of the enhanced mass transfer. However, this is not applicable for all macromolecules. For example, polyethyleneglycole (PEG) in aqueous solution shows a better resolution at lower temperatures.

Figure 4 summarizes several effects and shows how the optimization of particle size, number of columns, flow-rate and temperature can dramatically increase the resolution. Here a Dextran oligomer has been analysed using different columns and conditions. Best resolution is obtained with a low flow-rate on two 5 μm columns.

Practical Advice for Column Selection and Resolution Optimization

Use the lowest particle size possible. Small particle sizes can be used without any

restrictions especially for oligomers or proteins.

If analysis time is not an issue try lower flow-rates. This will not influence the amount of solvent needed for the analysis. Add columns of the same porosity to gain resolution. Add columns with other pore sizes to increase the separation range.

Ask column manufacturers for mismatch free column combinations. Do not combine linear/mixed-bed/multi-pore columns with single porosity columns to increase the resolution in the low molar mass area and the separation range. Don't confuse peak shoulders resulting from mismatch with higher resolution.

References

1. G. Reinhold, *The Column*, **17**(5), 7 (2009).
2. T. Hofe, *The Column*, **4**(4), 20 (2008).

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