Tips & Tricks: GPC/SEC BEWARE of Mismatch

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Can I tell if a chromatogram's shoulder or side peak is part of my sample?

Is it superior GPC/SEC resolution or artefact?

Resolution is the separation power of a column system that ideally results in well-defined peaks separated at the baseline. This is possible with a large number of pores in a wide range of sizes within the GPC/SEC column set.

To get more sophisticated and detailed information about sample shoulders or side peaks, the GPC/SEC resolution has to be improved, the separation range has to be increased, and the possibility of column mismatch has to be ruled out.

Mismatch is a "shoulder" or side peak that does not result from the physical reality of a molar mass distribution in the sample, but from uneven pore sizes distributions.

How is the GPC/SEC resolution improved and the separation range increased?

Two different column concepts are established in GPC/SEC separations: single porosity columns with narrow pore size distribution (mono-modal) and the linear or mixed bed columns with broad or multimodal pore size distribution.

For a given elution volume range ΔV a linear column clearly covers a wider molar mass range than a comparable single porosity column; however, the mono-modal column clearly shows a higher resolution (better separation) within the covered molar mass range (see Figure 1).

An appropriate combination of mono-modal columns will effectively expand the molar mass separation range, while maintaining the high resolution level. By combining narrow pore size distribution columns of different porosities, the high separation power is safeguarded so that polymers are baseline separated [resolution (R_{sp}>3)] over several molar mass decades. This is possible because there is a sufficient large number of pores in a wide range of sizes, providing a sophisticated and detailed GPC/SEC.

Figure 2 shows the difference in performance between a linear column and a combination of three single porosity columns.

Figure 1: Comparison between the separation range of a linear column (black curve) and a single porosity column (blue curve).

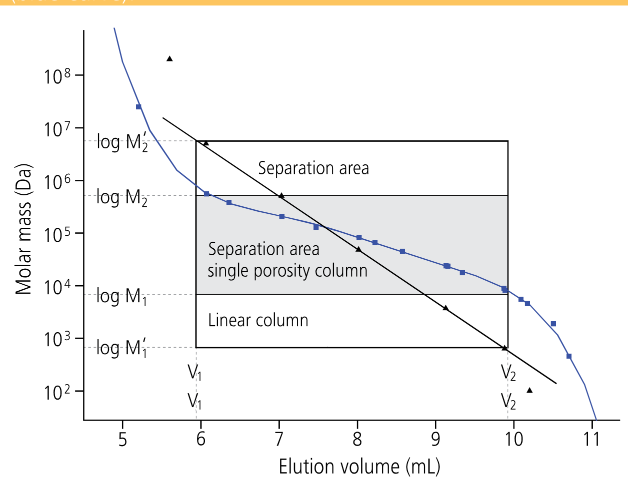

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Figure 2: Comparison between the calibration curves and separation performance of a PSS SUPREMA linear column and a combination of three SUPREMA columns (10 µm, 8 × 300mm) 100 Å + 1000 Å + 3000 Å; Dextran and PEO (ReadyCal Black, black line) samples are compared.

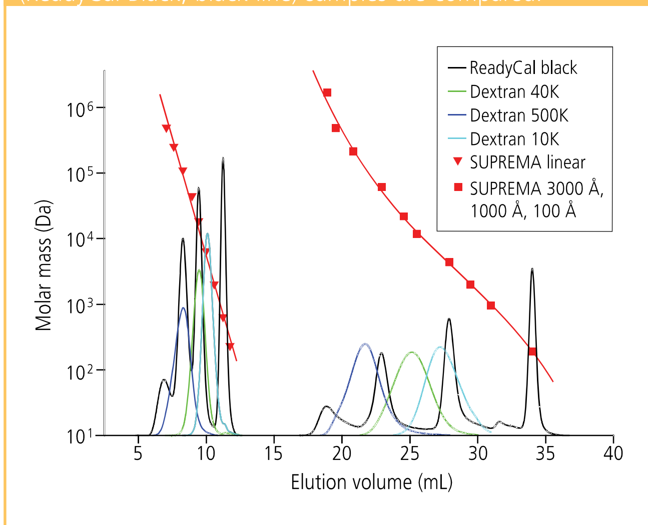
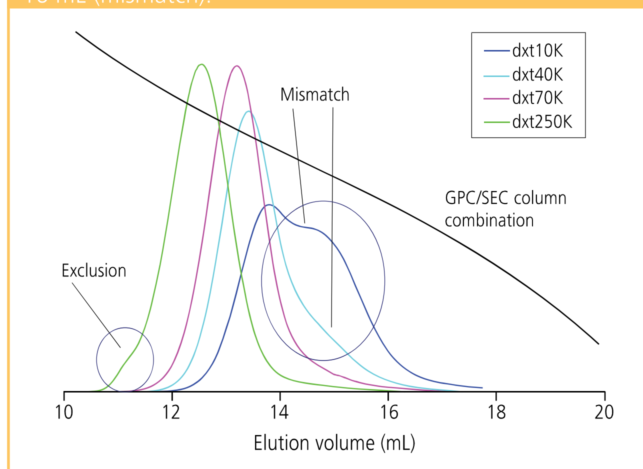


Figure 3: Example of column mismatch and high-molar mass exclusion using other supplier's column combination (SEC analytical columns, 10 µm and 30 cm length each). The chromatogram of dextrans are characterized by shoulders at 11 mL (exclusion) and between 14 mL and 16 mL (mismatch).



The column combination leads to a clearly better resolution across the entire molar mass (respective elution volume area) and shows a larger separation range. With a polymer mixture of four different molar masses (PSS PEO ReadyCal Black), the four polymers are well separated at the baseline.

One linear column yields results that are adequate for product screening three times faster than the analysis time with the column combination. A combination of three similar linear columns could improve the resolution but not the separation range.

In principle:

- combining two columns of the same porosity improves the resolution (R_s) by a factor of 1.4
- combining different porosities increases the possibility of a column mismatch.

Why column mismatch has nothing to do with a high resolution

The fundamental condition for a column combination of different single porosities is a homogeneous pore size distribution. This is also true for linear columns but almost impossible to achieve.

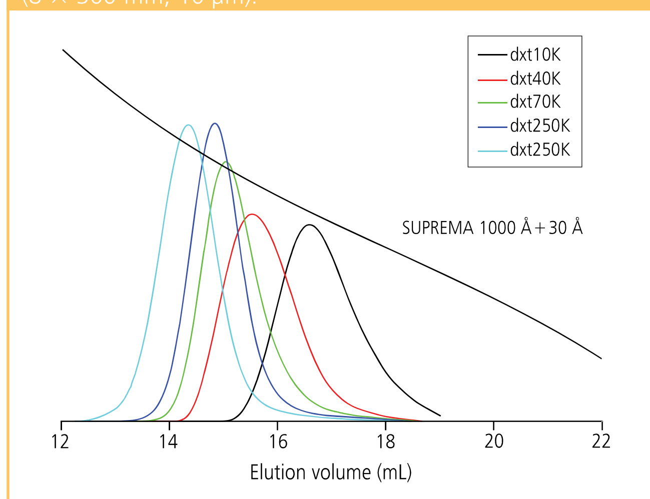
The different pore sizes have to match together. If the difference of the pore size distribution of two single porosities is too large (i.e., 100 Å and 1×10^6 Å) there is a gap of pore sizes at certain elution volumes leading to a lack of resolution in the area. The calibration curve should reveal a mismatch through a sudden slope change. The first derivative of the calibration curve should point towards the instability. The chromatogram of a given sample would show a shoulder (multi modality), in the area of instability.

The opposite is also true; if too many pore sizes occur in the overlap range, the same kind of problem is caused.

Generally speaking, the column mismatch effect is a local fluctuation of the resolution of a column (or fluctuation of the number of pores and pore sizes within a certain range), leading to chromatogram artifacts (shoulder). Therefore, the "shoulder" does not result from the physical reality of a molar mass distribution in the sample. The column mismatch shoulder disguises as higher resolution.

A true column resolution must be strictly differentiated from a pore size mismatch masked as resolution.

Figure 4: Column combination without mismatch and without high molecular exclusion exemplified by a combination of SUPREMA columns 30 Å + 1000 Å (8 × 300 mm, 10 μm).



How to distinguish a column mismatch?

Often, the discussed inconsistencies are so small that the calibration curve, the slope of the calibration curve and the first derivative do not point to the possible column mismatch for various reasons:

- The narrow-distributed polymer reference (calibration) standards are too narrow to reflect the column mismatch properly.
- The polymer concentration in the slices of the chromatogram is too high.
- The mismatch area is between two calibration points and, therefore, undetected by the GPC/SEC calibration fit function.

The most succcesful and most sensitive way to prove a column mismatch is an appropriate chromatographic test with suitable reference substances. Various homogeneous and symmetric broad-distributed reference standards with different molar mass average values should be used.

Figure 3 shows different broad-distributed Dextrans on an established GPC/SEC column combination of a column supplier. The calibration curve is smooth and shows no reference to a column mismatch. Some chromatograms, however, are characterized by shoulders.

Two effects can be detected: exclusion and mismatch. The high-molecular exclusion occurs at approx. 11 mL; the calibration curve here runs very steeply; a shoulder appears in the peak because no pores are available for the separation. The shoulder at 15 mL is caused by a column mismatch (i.e., uneven distribution of pore sizes).

On a comparable PSS SUPREMA column combination 1000 Å + 30 Å the same dextrans elute smoothly and without shoulders. Mismatch and high-molecular exclusion do not arise here (see Figure 4).

Summary

- Single porosity columns present high resolutions and small separation ranges.
- Linear-columns have large separation ranges with poor resolutions.
- With column combinations the analysis can be improved.
- Side peaks or shoulders of broad-distributed samples may be possibly attributed to an incorrect column combination or a resolution lacking column.
- Column mismatch can be detected by broad-distributed standards.
- Column mismatch cannot always be deciphered from the calibration curve.

References

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