

Tips & Tricks: GPC/SEC Copolymer Analysis in GPC/SEC 3: Two-dimensional Chromatography

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Copolymer analysis is challenging due to the effect that at least two distributions can be present simultaneously: the molar mass distribution (MMD) and the chemical composition distribution (CCD). A problem in GPC/SEC, that cannot be solved by using any detection technique, is that the method may suffer from limited chromatographic resolution.

Combination of different separation techniques into a single experiment (multi-dimensional chromatography; also called 2D chromatography, orthogonal chromatography and cross-fractionation) can help to overcome this. The corresponding peak capacity in an n -dimensional separation is substantially higher because each dimension contributes to the total peak capacity as a factor and not as an additive term.

For copolymer analysis (e.g., block copolymers, graft copolymers or others), the most common approach is to combine LAC (Polymer-HPLC, separation according to CCD) or LAC under critical conditions

(separation according to CCD or end groups) with GPC/SEC in the second dimension to investigate the molar mass distribution MMD.

Figure 1 shows the advantages of 2D method combinations. They can show sample differences that cannot be detected by analysis in individual separation methods. For all four samples both separation techniques show no difference in their chromatograms. Only on-line two-dimensional chromatography provides users with the entire picture.

What is needed for 2D Chromatography?

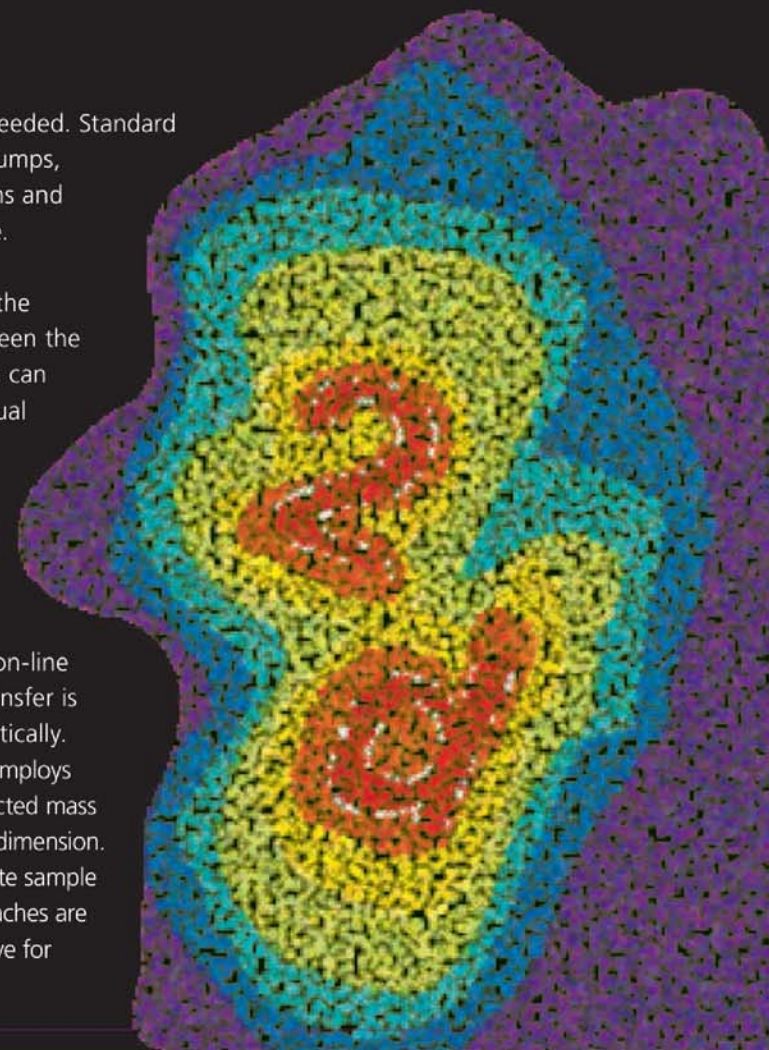
In two-dimensional chromatography two different separation techniques are combined; for example HPLC, LACCC, GPC/SEC, TREF, IC, CE, GC or others. A sample is separated using one technique and the sample fractions are then analysed using the second technique.

To perform a 2D analysis the components (and methods) required for each single

separation technique are needed. Standard instrumentation, such as pumps, autosamplers, column ovens and detectors can be used here.

The pivotal part in any two-dimensional set-up is the fraction transfer step between the separation methods, which can be on-line or off-line, manual or automated. Off-line systems require a fraction collection device with manual transfer and reinjection of the first dimension fractions into the second instrument. In on-line 2D systems the fraction transfer is preferentially done automatically.

Comprehensive 2D work employs complete transfer of the injected mass from the first to the second dimension. This ensures that the complete sample is analysed. Heart-cut approaches are not considered comprehensive for obvious reasons.



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The advantages and limitations of the different off-line and on-line approaches are summarized in Table 1.

Different set-ups and approaches for two-dimensional analysis are described in the literature.¹

Obviously, two-dimensional separations take more time depending on the number of transfer injections from the first into the second dimension. Time requirements can be substantial if analytical GPC/SEC columns will be used in the second dimensional separation. However, the availability of high throughput and HighSpeed GPC/SEC columns allows for much faster 2D results. If the transfer is on-line, for example with a dual loop valve,

two-dimensional chromatography can be fully automated and the time to analyse a sample can be decreased to 2–3 hours.

Very important in 2D chromatography is data presentation and analysis. Comprehensive 2D experiments yield a three-dimensional data array (similar to DAD/PDA data sets) of data tuples in the form of [(dimension 1 property), (dimension 2 property), (concentration)] which can be expressed in many ways.

3D surface plots generated from a lattice plot by interpolation, visualize results, however quantification is not possible. They can be regarded as virtual 3D landscapes that can be viewed from any observation position in space and allow users to investigate the peak

shape or trace amounts on the back side of major peaks (see also Figure 2). Contour plots (or contour maps) are two-dimensional representations of the 3D surface plots. Different concentrations are normally shown as different colours. The chromatographic axis of the first dimension is plotted on the ordinate, while the properties determined in the second dimension are shown on the abscissa. Here quantification is possible. The CCD can be determined via an appropriate calibration

with samples of known composition (**see the previous Tips & Tricks**). The MMD can be calculated based on a conventional molar mass calibration of the second dimension.²

Figure 3 shows a 2D contour plot as well as the corresponding first and second dimension LAC and GPC/SEC traces.

Practical advice for 2D

In general, performing a 2D experiment is often easier than expected. The major work

Figure 1: Two peaks in GPC/SEC and LAC can be interpreted in different ways when the samples are heterogeneous in composition and molar mass. A few examples for possible solutions are shown.

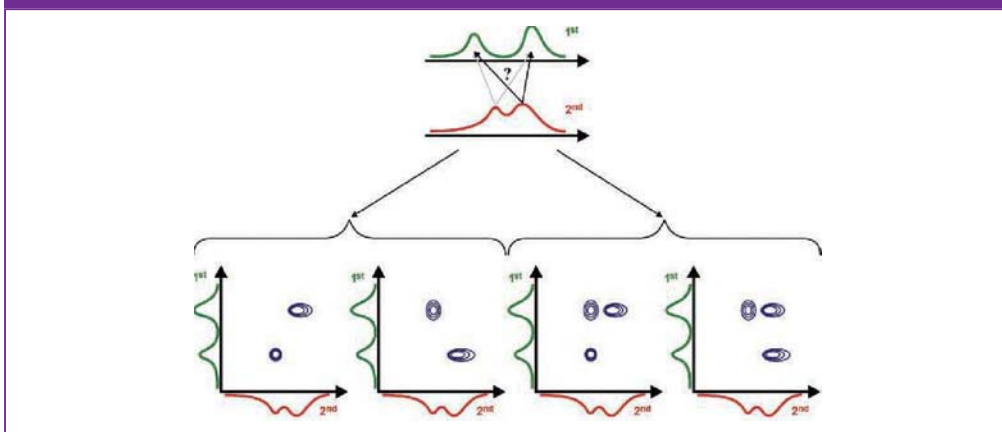


Table 1: Comparison of 2-dimensional transfer options

Transfer	Mode	Advantages	Disadvantages	Example
Manual	Off-line	<ul style="list-style-type: none"> • Very simple • Fast set-up 	<ul style="list-style-type: none"> • Time-consuming • Not for routine work • Not precise • No correlation of fraction elution to transfer time • Not quantitative 	Test tubes, manually exchanged
Automatic	Off-line	<ul style="list-style-type: none"> • Simple • Easy • Fast set-up 	<ul style="list-style-type: none"> • Less precise • No correlation of fraction elution to transfer time • Not quantitative 	Fraction collector, storage valve
Automatic	On-line	<ul style="list-style-type: none"> • Correct concentrations • Correct transfer times • Automation 	<ul style="list-style-type: none"> • Transfer not quantitative • Setup time 	Injection valve with single-loop (with actuation)
Automatic	On-line	<ul style="list-style-type: none"> • Correct concentrations • Correct transfer times • Quantitative transfer • Automation 	<ul style="list-style-type: none"> • Setup time • Special valve required 	Dual-loop valve with <ol style="list-style-type: none"> 1. actuated 8-port valve 2. combination of two conventional 6-port injections valves

is to develop robust methods for both dimensions. Commercial tools are available for the transfer from one dimension to the other and the result presentation and evaluation.

The proper sequence of separations methods is important to achieve highest resolution and accurate determination of distributions. It has been shown that it is best to apply the method with the highest selectivity for a property as the first dimension. This ensures the highest purity of eluting fractions are transferred

into the subsequent separation. Obviously, techniques such as GC and SFC, which destroy the mobile phase, can only be used as the second dimension. For the characterization of copolymers mostly LAC or LACCC has been hyphenated with GPC/SEC, but also other combinations have been applied successfully.

The compatibility of mobile phases that are transferred between chromatographic dimensions is an important issue in designing two-dimensional experiments. Complete miscibility of the mobile phases

used in all dimensions is an obvious necessity. Otherwise the separation in the second method is dramatically influenced and the fraction transfer is restricted or completely hindered. In gradient systems, this requirement has to be verified for the total composition range.

In GPC/SEC dimensions, the transfer of mixed mobile phases can affect molar mass calibration. In order to get proper molar mass results, the calibration curves have to be measured using the extremes of mobile phase composition and tested for changes in elution behaviour and pore-size influence in the GPC/SEC column packing.

It has been shown to be advantageous to use the GPC/SEC eluent as one component of the mobile phase in the previous dimension to avoid potential interference and mobile phase incompatibility.

The best detectors for GPC/SEC dimensions are DAD/PDA detectors (if chromophores are available) or ELS detectors, due to the high sensitivity of these detectors and the baseline stability. RI detection is possible but sometimes these detectors are not sensitive enough or the chromatograms show strong solvent peaks at high elution volumes.

Figure 2: 3D surface plot for visual inspection and the results for the four identified peaks.

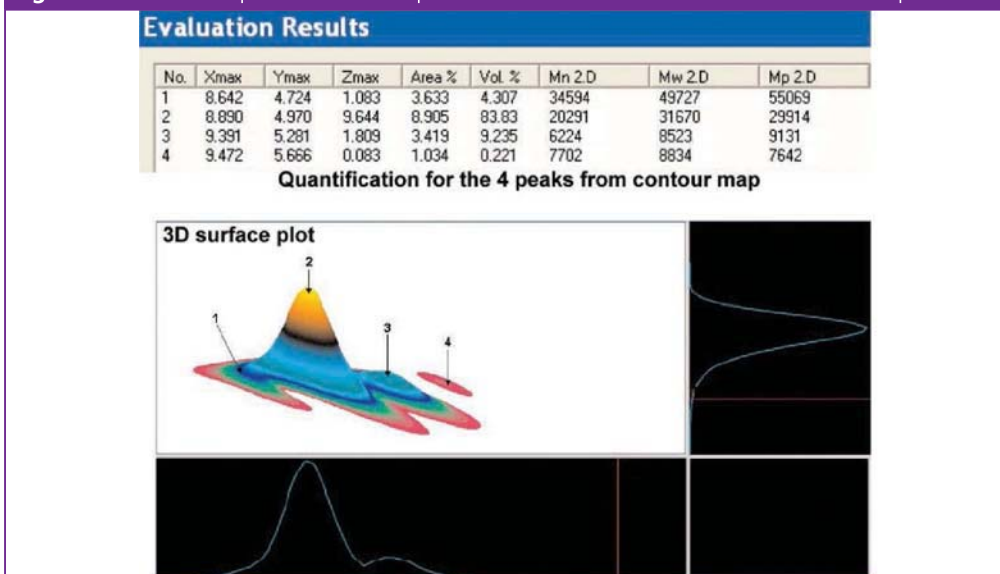
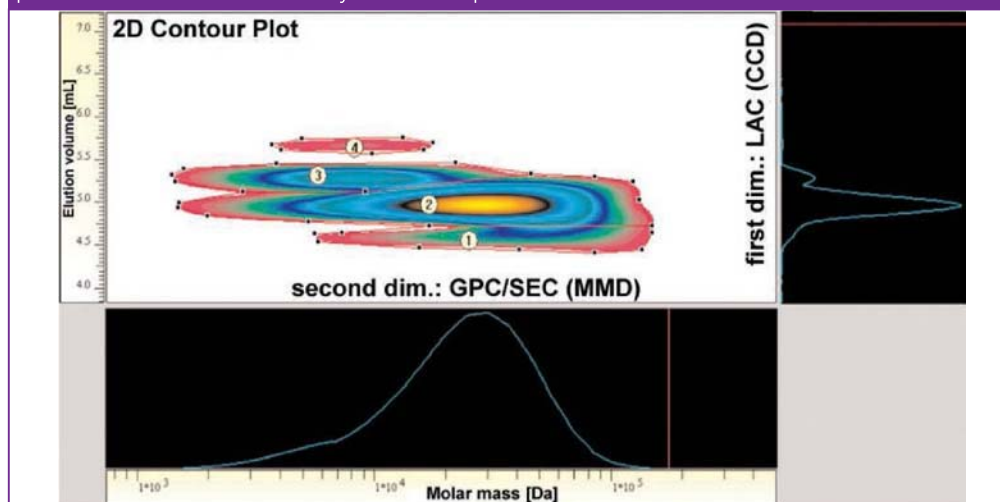


Figure 3: Example for a 2D contour plot with 4 different peaks where LAC shows only 2 peaks and GPC/SEC shows only one broad peak with shoulders.



What are the results for 2D chromatography of copolymers?

A famous example showing the high peak capacity and the possible results with 2D chromatography is the separation of polystyrene-polybutadiene block copolymers with different composition, molar masses and structure.³ Here a mixture of 4 copolymers with 20, 40, 60 and 80% butadiene is analysed. In addition to the different composition, there are two linear structures (PS-PBd, PS-PBd-PbD-PS) and two branched

structures, a 3-arm star with PS-PBd copolymer arms and a 4-arm star with PS-PBd copolymer arms.

This 16 component mixture has been analysed using LAC on a silica phase 5 μm 60 \AA column with a gradient iso-octane/THF (20% THF linear to 100% THF) followed by GPC runs on a SDV styrene-divinylbenzene phase in THF. Figure 4 shows the resulting contour plot and the results. Both dimensions have been calibrated so that it is possible to determine the molar mass distribution and

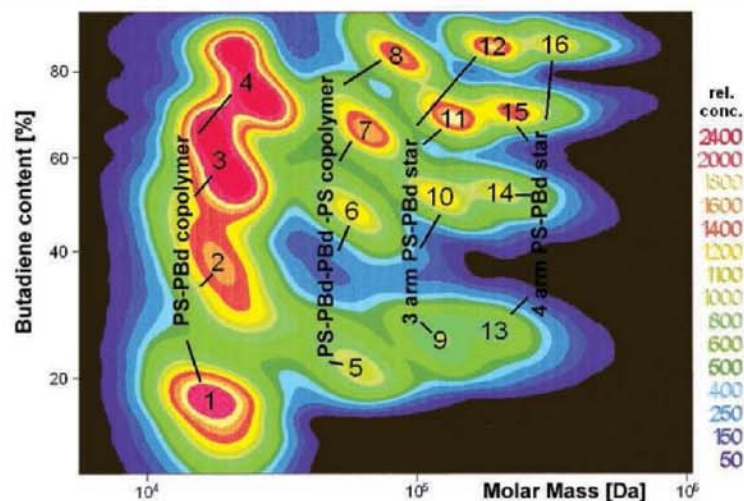
the chemical heterogeneity for each single peak. The butadiene content axis and the molar mass axis are shown for all peaks. The colour code gives additional information about the concentration.

References

1. H. Pasch, *Chapter 7: Two-Dimensional Liquid Chromatography*, in H. Pasch, B. Trathnigg, HPLC of Polymers, Springer-Verlag Berlin Heidelberg New York, USA (1998).
2. D. Held, *The Column*, 5(6), 18–21 (2008).
3. P. Kilz et al., *ACS Adv. Chem.*, 247, 223–241 (1995).

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Figure 4: Entire picture of a mixture of polystyrene-polybutadiene block copolymers with different molar masses and structures and different polybutadiene content. Up to 16 different species could be identified and quantified. Peak 1 is a linear PS-PBd copolymer with approx. 20% butadiene content, peak 16 is a 4-arm star polymer with approx. 80% butadiene content.



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