Comprehensive 2-Dimensional Liquid Chromatography for Polymer Applications

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OVERVIEW

As technology becomes increasingly more advanced, R&D and analytical processes across the many industries driven by materials sciences must also pivot to apply more advanced techniques that keep up with both market and consumer demand. Many of the applications applied in modern analytical chemistry including environmental analysis, food analysis, analysis of biological material comprising biopolymers and low molecular weight compounds, require effective chromatographic separation techniques that meet the continuously increasing demand for methods which enable the analysis of increasingly more complex samples, especially at low concentration levels.

Thoroughly understanding macromolecular structure is a fundamental aspect for the use of polymers in increasingly specific applications particularly because various polymers may vary in molecular weight, chemical composition, end groups, and architecture. Furthermore, most classes of macromolecules. Especially polymers, can exhibit more than one property distributions at the same time. This heterogeneity of polymers can make separation efforts incredibly difficult – especially when analyzing copolymers, additives, and other complex structures. Figure 1 below illustrate the complex distribution of homo and copolymers.

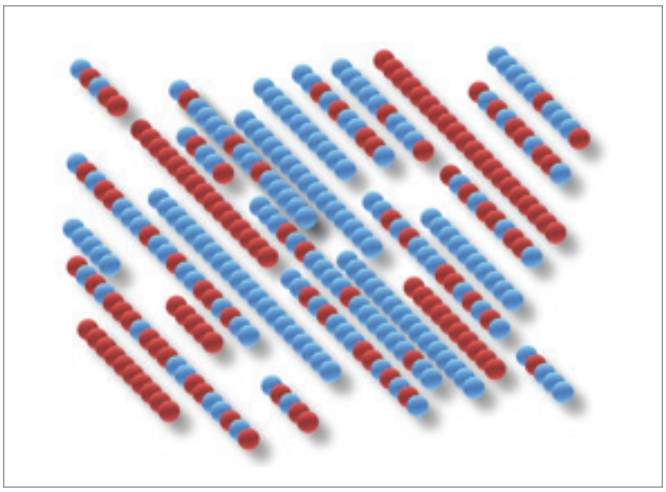

Figure 1. Illustration of homo and copolymers and their distribution.

While using traditional polymer analysis methods such as infrared spectroscopy (IR) or nuclear magnetic resonance (NMR), allow researchers to determine the type and concentration of monomers or functional end groups in a polymer, they do not provide the needed visibility into the differences in distribution of monomer units or functional groups among the chains.

Size Exclusion Chromatography (SEC) also referred to as Gel Permeation Chromatography (GPC), is considered the go-to analytical technique for polymer chemists and material scientists, with both molecular weight and polydispersity influencing the bulk properties of the resulting materials. A growing demand for advancement in the materials sciences calls for an improvement in both LC technologies and separation methods to overcome the challenges experienced with traditional SEC; which often suffers from limited peak resolution, low peak capacity, convoluted peaks, co-elution of species, and difficulty identifying impurities in the sample. It is particularly important to note that when separating by chemistry alone, co-elution of macromolecules with identical composition, but with different molecular weights, is simply not enough to analyze species of a more complex nature. Obtaining a more thorough and accurate understanding of complex polymeric structures is best achieved by separating samples through two distinct separation dimensions – first by chemistry and then by size. The combination of the results obtained through these two analyses significantly enhances the identification of components in complex polymer samples such as co-polymers and polymer blends.

Two-dimensional liquid chromatography (2D-LC) provides an ideal alternative for the analysis of complex samples owing to its improvement in both separation selectivity and peak capacity. There are three main modes of multidimensional or 2D separation; heart cutting, “trap and elute”, and chromatography. In heart-cutting 2D chromatography, a sample is separated in the 1st dimension column, followed by isolation (or cutting out) of specific unresolved peaks to be injected into a 2nd dimension column for further separation. Heart-cutting is generally used for less complex samples. Similarly, “trap and elute” involves trapping an analyte of interest in a trap in the 1st dimension column and then washing it to remove any impurities. Next, a secondary pump applies a gradient to the column transferring the analyte of interest to the 2nd dimension column for separation. Comprehensive 2D chromatography involves a complete and immediate transfer of the sample from the 1st dimension to the 2nd dimension without significant loss of the 1st dimension resolution and offers the best solution for multidimensional separation of complex polymer samples. Comprehensive 2D chromatography employs a combination of two different columns and separation modes between the 1st and 2nd dimensions as noted in figure 2 below.

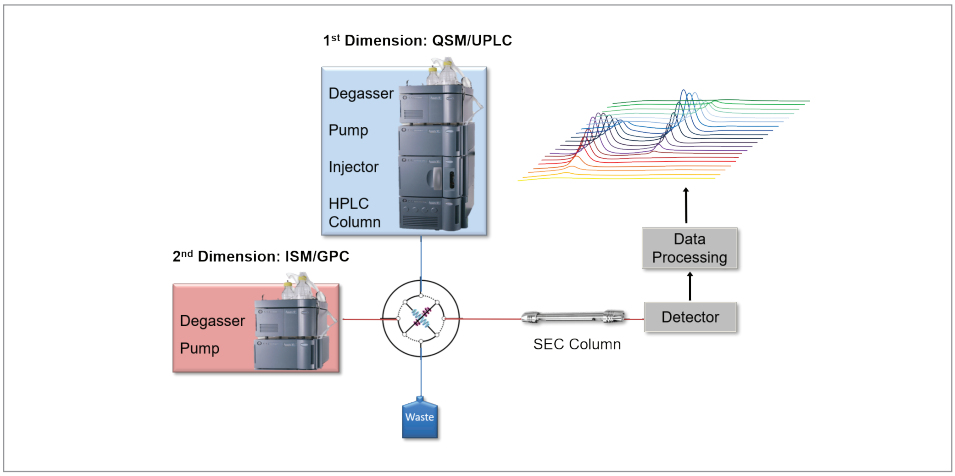

Figure 2. Separation scheme of a two-dimensional liquid chromatography experiment.

In comprehensive 2D chromatography, independent separation modes are combined to achieve a higher separation power. This means that the overall peak capacity of the 2D-LC system is equal to the product of their peak capacities, as illustrated in the equation below where N denotes peak capacity;

$$N2D = N1 \times N2$$

This equation is valid only, if the implemented retention mechanisms in these two columns are completely independent, so that the entire separation space is used. It also considers that there is no loss of the 1st dimension peak capacity due to back mixing during the transfer of the effluent to the 2nd dimension.

The peak capacity achieved in multidimensional separations compared to the time that is needed scales quite differently in 1D versus 2D separations. 1D separations use long, slow gradient elutions and peak capacity increases slowly towards an overall limit. The separation efficiency of any single separation method is limited by the efficiency and selectivity of the separation mode such as the plate count of the column selected. Simply adding more columns will not allow an analyst to separate more components in a complex sample, due to the limitation of peak capacities.

A demonstrative example of the superior separation capability of 2D experiments are noted when analyzing graft copolymers. Since syntheses rarely yield a 100% conversion rate, 2D analysis can be helpful in separating the architectural product with different grafting density from non-grafted side chains. This example is illustrated in Figures 3a and 3b where it is clearly visible that a 1D analysis would not sufficiently separate the reaction mixture.

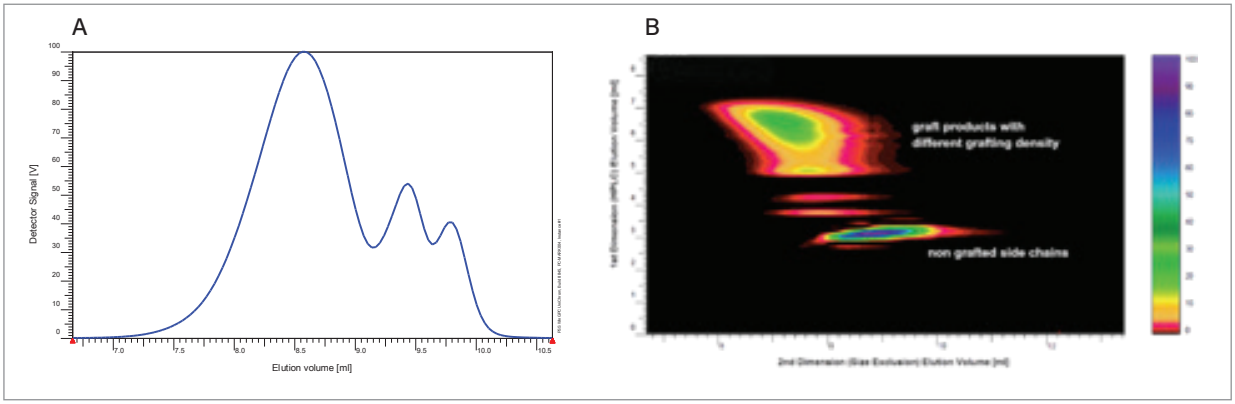

Figure A and B: SEC RI data for a graft copolymer and the corresponding 2D comprehensive separation.

The goal in the characterization of copolymers with such a bivariate distribution is to construct a 2D map where the results of the first separation dimension is exclusively sensitive to molecular weight distribution with the second separation dimension offering insight into the chemical composition distribution. Together, the information provided by comprehensive 2D chromatography offer analysts a new and exciting insight into their polymer samples and offers clear guidance into the next steps in the polymer R&D process.

COMBINATIONS OF 2D SEPARATION

2D chromatography is not limited to a specific mode of separation. Different separation modes such as RP, NP, SEC, LCCC, HILIC, LAC, to name just a few, can be combined to construct a 2D-LC method according to the sample properties. Possible combinations include: LC x SEC, LCCC x SEC, RP x RP, RP x NP, HILIC x RP, HILIC x HILIC. The table below contains examples of possible combinations.

Application	Type	MP	Configuration	Reference
		Natural Polymers		
Na CMC	LAC x SEC			Carbo Polym 130 (2015) 77
		Rubber		
SBR and butyl rubber	HPLC x SEC	Chloroform-cyclohexane 0:100 (v/v)		Heinz LC, Siewing A, Pasch H. E-Polymers. 2003
Ethyl methacrylate- and styrene-grafted Epoxidized natural rubber	LCCC x APC			Graef, S. M., 2003, J. Appl. Polym. Sci., 88: 2530-2538.
Styrene-butadiene rubber	LCCC x SEC		QSM x ISM	PSS
Analysis of TPE	UPLC x SEC		QSM x ISM	PSS
		Co-Polymers		
PS-b-PI	NP-(TGIC) x RP	isoctane/THF 97/3	QSM x QSM	PSS
Graft copolymers (Also acrylates)	LCCC x SEC			Muller, ACS synposium series, vol 768
PEG-g-PVAc copolymers	LC x SEC			J Chrom A 1130 (2006) 43
		Coatings		
Coatings	LCCC x SEC		QSM X ISM	PSS
Coatings (PU)	UHPLC X UHPSEC(HILIC)	H2O/THF 25-95% THF 0.1% FA		Anal. Chem. 2012, 84, 7802−7809
		Polyacrylate		
Acrylates	RP x SEC	ACN/THF	QSM x ISM	Raust JA, J Chromatogr A, 1203:207
Acrylates PMMA, PBMA and P(MMA-co-BMA)	UHPLC X UHPSEC	ACN/THF 15.5-80% THF	BSM	Anal. Chem. 2012, 84, 7802−7809
		Surfactants		
Surfactant from renewable resources	IEC x SEC	E	QSM x ISM	PSS
Complex surfactant mixtures	NP x RP	H2O - ACN 0.1% TFA	micro pump	Anal Chem 75, 3, 2003; 373
PEG & surfactants				Anal. Chem. 70 (1998) 1585
Alkyl-ethoxylates	NP x RP		QSM x QSM	PSS
		Oil		
By-products in engine oil	LCCC x SEC		QSM x ISM	PSS
H-PS/D-PS blend	LCCC x SEC	Polystyrene THF–ACN49.5:50.5(v/v)		J Chrom A 1265 (2012) 95
Branching (star branched PS)	LAC x SEC			PSS

Combination of interactive UHPLC with ultra-high pressure size exclusion chromatography (UHPLC X UHPSEC) are especially useful for co-polymer analyses. Online comprehensive LC × SEC can offer much higher peak capacities than 1D separations. The main reasons why LC × SEC is the most commonly used approach for polymer analysis is because the combination of the two offers excellent dimensional compatibility, there are high degrees of orthogonality of the two techniques, and the possibility to save valuable time due to the relatively fast runtime associated with performing SEC. With UHPLC × UHPLC, separations of industrial polymers can be achieved within 1 hr.

INSTRUMENTAL SET-UP FOR COMPREHENSIVE 2D

The separations in the individual dimensions of 2D systems are controlled by the same rules as those used in the separations in 1D systems. Figure 4 displays a set-up schematic for a 2D experiment.

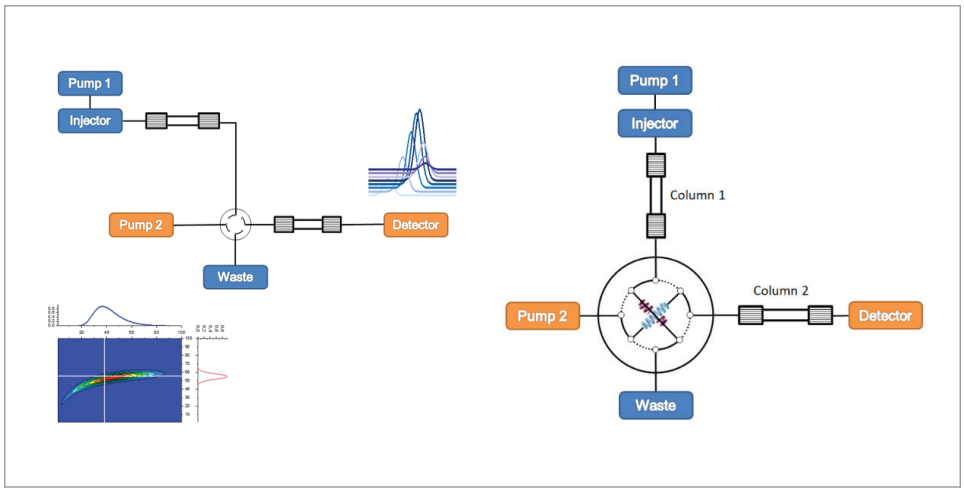

Figure 4. Instrumental setup for 2D.

Both dimensions consist of commonly equipped LC systems with pumps and column managers whereas only the 1st dimension utilizes a sample manager. Detectors are usually found in the 2nd dimension and are typically optional in the first dimension. Gradient elution is conducted in the 1st dimension. Since the dwell time of a gradient capable pump is larger than that of an isocratic pump, the gradient capable pump is included in the 1st dimension to avoid interference with the separation in the 2nd dimension. The supported pump configuration for 2D Advanced Polymer Chromatography follows: APC 2D = quaternary solvent manager (QSM) x isocratic solvent manager (ISM). 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.

The two dimensions are connected on-line through an interface, such as a eight-port or a ten port switching valve or two six-port switching valves in series. The valve is equipped with two sampling loops of identical volume. Effluent from the 1st dimension is collected in one of the loops while the content of the other loop is transferred onto the 2nd column for separation. At the same time, the first loop is being filled by a new effluent fraction. The two loops are regularly switched between the collecting (A) and the elution (B) positions in an alternating valve operating cycle. Hence a single component may be divided across two or more 2D fractions. Fractions collected by the detector eluting from the 2nd dimension are stacked side-by-side as can be seen in Figures 5A–D.

Having a column manager in the 2nd dimension means that all fractions within the comprehensive 2D separation are recorded by the 2nd dimension detector. Dilution of the analytes in each stage of a 2D separation in addition to high 2D flow rate, can in some cases pose challenges with detection. Commonly used detectors for 2D analysis include evaporative light scattering detectors (ELSD), fluoresence, and diode-array UV-Vis detectors; that can go down to the nano-gram level, alleviating problems with both dilution and high flow rates. The use of mass spectrometers as a detection method is also reported in the literature, but has limitations in 2D polymer separations due to the ionizability of larger sized fractions.

2D data is presented in a 2D plane or contour plot as noted in Figure 5 below.. A contour plot displays a 3D surface on a 2D plane consisting of all 2nd dimension injections correlated to their 1st dimension elution time/volume. The contour plot easily highlights individual peaks that cannot be otherwise seen in any of the 2 dimensions individually. For the identification of individual compounds in the chromatograms, an appropriate algorithm is applied to transfer the read-out of the peaks and valleys from the data matrix file to a 2D diagram. The approach allows for the identification of overlapping peaks within the fractions from the first dimension, so that a single maximum can be assigned to each peak in the 2D retention plane. The 2 chromatograms are then related by injection time, so that quantitative information can be obtained from peak areas/volumes.

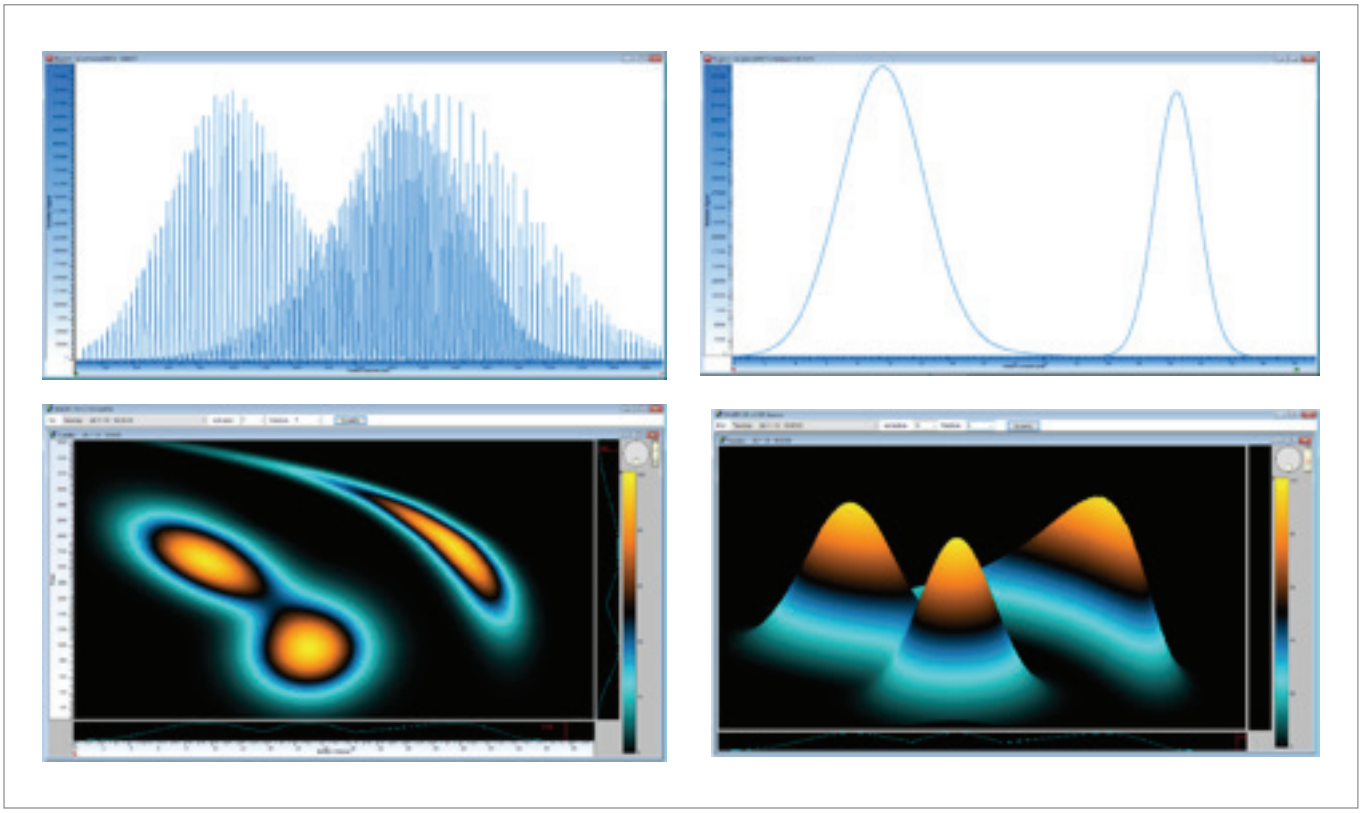

Figure 5A–D. Examples of typical 2D chromatograms and contour plots.

The choice of columns to use in each dimension requires careful thought. Generally, it is important to remember that a low flow rate is needed in the 1st dimension, followed by a high flow rate in the 2nd dimension. According to literature reviewed, the use of a micro/narrow bore column in the 1st dimension and a larger bore column in the 2nd dimension is recommended to accommodate very short analysis times for specific applications.

For gradient-elution, UHPLC allows relatively high sample loadings, so that the impact on detection sensitivity following two successive dilutions in a 2D experiment can be reduced. The use of a sub-2 µm particle column in the first dimension can provide greater efficiencies (peak capacities) in comparison to conventional HPLC. The resulting peak capacity of the 2D separation is an order of magnitude larger than that of a 1D separation performed. Thus, most separations with analysis times less than 2 hrs and peak capacities between 500 and 1000 can be achieved.

CONCLUSION

As R&D becomes more complex, the need for multi-dimensional separations is becoming more prevalent, especially for analysis of both co-polymers and architectural polymers.

Comprehensive 2D separations offer the most suitable option for 2D polymer analysis. Using 2D separation techniques can offer analysts huge improvements in both separation selectivity and peak capacity.

There are a wide range of combinations and configurations possible with 2D chromatography. When setting up your 2D chromatography system configuration, careful thought should be given to specific method development needs, desired outputs, and system components required. With a well-thought-out method development approach however, analysts can gain significantly more INSIGHT into the various aspects of polymer samples, which would not otherwise be available.

References

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