Multi-Dimensional Separations of Polymers

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Multi-Dimensional Separations of Polymers

Synthetic polymers and comprehensive two-dimensional liquid chromatography (LC × LC) are a synergistic combination. LC × LC provides unique insights in mutually dependent molecular distributions. Synthetic polymers offer clear demonstrations of the value of LC × LC.

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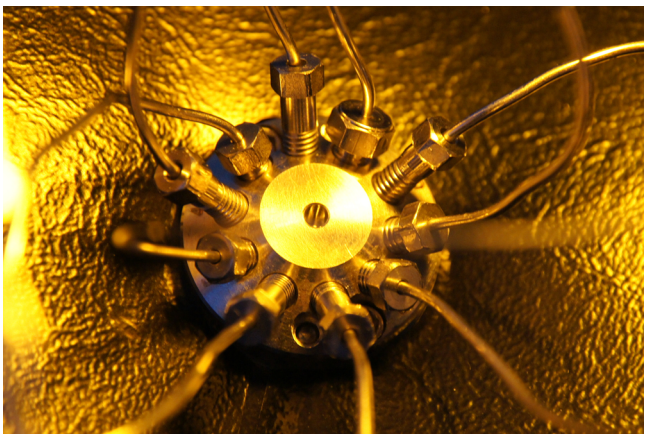

Petra Aarnoutse

Synthetic polymers (and many natural ones) have a relatively simple molecular structure. They often consist as linear chains of monomers (R). The structure of such macromolecules can be described by $X(R)_nY$, where X and Y are the end groups and n is known as the degree of polymerization. For a given polymer, with given end groups, the molecule is characterized completely if we measure n. There are a number of ways to measure the *average* degree of polymerization. These include colligative properties (such as osmotic pressure), light scattering, viscometry and, for low-molecular-weight polymers, spectroscopic techniques, such as NMR. However, many of the properties of polymers are strongly affected by the variation in molecular weights. Knowing the average molecular weight does not usually suffice. When you are contemplating wading through a river it helps little to know that it is one meter deep, on average.

Enter separations. Separation techniques are indispensable if we wish to characterize the molecular distributions of polymers. The concept is simple. If we can separate the molecules according to the degree of polymerization, we can measure the amount present of each molecular weight (or each value of n), and from there we can calculate all the characteristic molecular-weights (number-average, weight-average, etc.; equivalent to the statistical moments of the distribution). The separation is akin to separating a homologous series of n-alkanes by gas chromatography (GC). However, the molecules are too big (and not sufficiently volatile) to be compatible with GC and we cannot separate all the different masses. For relatively low molecular weights (M_r < 10 000; M_r is the relative molecular weight equal to the absolute molecular weight divided by 1/12 of the weight of the ^{12}C isotope), the individual oligomers may be separated with mass-spectrometric techniques, such as matrix-assisted laser desorption/ionization (MALDI). However, MALDI fails for larger molecules and it is notoriously difficult to obtain quantitative information from signal intensities, because of selective-ionization, ion-suppression, and discrimination effects.

In separating polymers we usually have to be satisfied with an envelope of peaks; "a hump" with little or no structure. This hump is then translated into a molecular distribution by transferring the retention-time axis to a more-meaningful property, such as the molecular weight. In size-exclusion chromatography (SEC) the relationship between molecular weight and elution time (or elution volume) is known as a calibration curve.[1] SEC (based on exclusion from pores in the stationary phase) and hydrodynamic chromatography (HDC, based on exclusion from the stationary-phase surface[2]) separate according to size. Such techniques are used to obtain molecular-weight distributions (MWDs). Other techniques can be used to separate polymers according to different aspects of the molecular structure.[3−5] Gradient-elution liquid chromatography (GELC) and temperature-gradient interaction chromatography (TGIC[6,7]) may be used to obtain chemical-composition distributions CCDs), if retention time can be converted to the ratio of different monomers present.

Not all polymer molecules can be described by the schematic structural formula $X(R)_nY$ introduced at the outset. The CCD mentioned above would be meaningful for a copolymer with structural formula $X(R)_n(R')_mY$, where R and R′ are two different monomers. If these are connected in the chain in a random order, we speak of statistical copolymers; if all R and all R′ are grouped in one or two long strings we speak of a block copolymer. Table 1 lists a number of molecular characteristics of synthetic and natural polymers that give rise to distributions. Techniques to determine the average values of these properties and techniques to separate a polymer sample according to the indicated property are also listed in the table.

It is possible to combine the techniques to measure averages (third column in Table 1) with separation techniques (last column). When two techniques from the same row in the table are combined, we obtain combined methods that may yield more-accurate distributions. For example, SEC may be used as a separation technique in combination with light-scattering

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Table 1. Molecular Distributions of Polymers and Associated Analytical Characterization and Separation Techniques

Molecular characteristic	Distribution	Techniques to measure averages	Separation techniques
Molecular weight	Molecular-weight distribution (MWD) or Molar-mass distribution (MMD)	Osmometry Light scattering Viscometry NMR (low M_r)	Size-exclusion chromatography (SEC; also known as Gel-permeation chromatography) Gel electrophoresis
Chemical composition (ratio of monomers)	Chemical-composition distribution (CCD)	Spectroscopy Pyrolysis-GC(-MS)	Gradient-elution liquid chromatography (GELC); temperature-gradient interaction chromatography (TGIC) Capillary electrophoresis (CE)
End groups or functional groups	Functionality-type distribution (FTD)	Spectroscopy, titrations (low M_r)	Isocratic or gradient-elution LC Capillary electrophoresis (CE)
Degree of branching	Degree-of-branching distribution (DBD)	Light scattering Viscometry NMR	Molecular-topology fractionation (MTF)
Tacticity	Tacticity distribution	NMR	Temperature-rising elution fractionation (TREF); Gradient-elution liquid chromatography (GELC)

and/or viscometry detectors.[8,9] The separation is based on molecular size, rather than molecular weight. The detection techniques serve to calibrate the system, generating a calibration curve (molecular weight vs elution time) during the analysis. Likewise, we may combine a spectroscopic detector with GELC to obtain accurate chemical-composition data online. This works well with a UV detector, but unfortunately many important polymers lack chromophores. The use of FT-IR[10] or NMR[11] spectroscopy for the purpose is much more complicated.

When combinations are created across the different rows, techniques are created that yield additional information on the sample polymer. For example, SEC can be combined with a spectroscopic detector to yield the *average* chemical composition as a function of molecular weight.[12] This allows diagnosing the presence (or absence) of a so-called "composition drift" in the polymer. SEC may be combined with light-scattering and/or viscometry detectors to characterize the *average* degree of branching as a function of molecular weight[9]

However, a distribution rarely comes alone. Very often we are confronted with composite mixtures and multidimensional distributions and, as was the case for one-dimensional distributions, polymer samples are not fully characterized when just *averages* of one of the key properties are obtained. A case in point is the distribution of functional groups in reactive (pre-) polymers. Molecules with two or more reactive groups may act as cross-linkers and thus add to the strength and rigidity of the final polymeric product. In contrast, molecules with one functional group will weaken the network, while molecules without functional groups will remain unreacted. Knowing the *average* number of functional groups per molecule does not allow us to predict the behavior of a cross-linking (pre-) polymer in a reaction mixture. An average number of 1 may correspond to a sample in which all molecules have a single functional group. This will result in a non-cross-linked polymer that may be dissolved or processed as a melt. An average number of 1 may also result from a 1:1 mixture of nonfunctional molecules and difunctional ones. Such a mixture may lead to a densely cross-linked, insoluble polymeric network. This example makes it clear that two-dimensional distributions cannot be fully characterized without two-dimensional separations.

COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

In two-dimensional (2D) separations, two different mechanisms are applied to separate a polymeric sample. Ideally, the retention times obtained in the two dimensions are completely independent, in which case the retention mechanisms are called "orthogonal". 2D separations can be performed in two different ways.[13] One is the "spatial" mode, in which the different components in a mixture end up in different positions in the chromatographic bed and are thus separated "in space". Common examples of spatial 2D separations include two-dimensional thin-layer chromatography (2D-TLC) and two-dimensional poly(acrylamide) gel electrophoresis (2D-PAGE). The main disadvantage of such separations is that detection must take place on the substrate, which precludes the use of the many detection systems that have been developed for column LC.

The second, more-common way in which 2D liquid-phase separations can be performed is the so-called comprehensive two-dimensional (column) liquid chromatography (LC × LC).[14−16] In this case a sample is first separated into many fractions on a first-dimension (^{1}D) column. Each of these fractions is subsequently separated on a second-dimension (^{2}D) column. The effluent of this ^{2}D column is monitored using one or more flow-through detectors. Thus, the different components are separated "in time". If all the relevant analytes reach the detector and the separation achieved in the first dimension is essentially maintained (despite the finite number of fractions), we speak of a comprehensive two-dimensional separation.

The recorded data are a series of ^{2}D chromatograms. A single component may be divided across two or more ^{2}D fractions. Algorithms are required to construct a comprehensive two-dimensional chromatogram and to combine peaks from different ^{2}D chromatograms into three-dimensional peaks.[17] Before this latter step, integration may be performed on the two-dimensional data before the areas (or heights) of peaks belonging to one component are added.

IMPLEMENTATION OF LC × LC FOR SEPARATING POLYMERS

The typical hardware required for LC × LC consists of two columns, two pumping systems (gradient or isocratic, depending

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on the requirements of the analysis) one 10-port 2-way switching valve and one (set of) detector(s).[18] The two-dimensional chromatograms are reconstructed from the string of one-dimensional chromatograms recorded by the detector.[17,19] A possible valve configuration is shown in Figure 1.

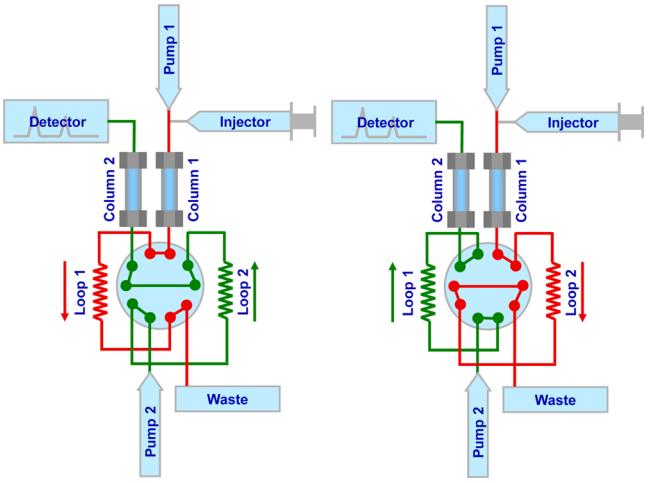

Figure 1. Possible valve configuration for LC × LC.

In this configuration fractions are sent to the ^{2}D column in back-flush mode. The difference in extra-column volumes between the two flow paths in the second-dimension must be minimized in this asymmetrical configuration.

Although it is feasible to stop the ^{1}D pump during the ^{2}D analysis, the most-convenient and time-efficient mode is to perform the ^{2}D separations while the first-dimension separation is taking place (in "real time"). This implies that the complete ^{2}D separation should be completed during the time in which one fraction is collected from the ^{1}D separation. If this is not the case, late-eluting compounds from the 2D run will appear in subsequent runs. This phenomenon is more commonly encountered in comprehensive two-dimensional gas chromatography (GC × GC), where it is known as "wrap-around". In in-line, real-time LC × LC, the ^{1}D separation is necessarily very slow and the ^{2}D separation very fast,[20,21] such that we find for the ratio of the analysis times in the two dimensions $^1t_{anal}/^2t_{anal}=n_f$, where n_f is the total number of fractions collected from the ^{1}D effluent. There are two ways to realize these vastly different analysis times. The ^{1}D column tends to be longer than the ^{2}D column, and the ^{1}D flow rate tends to be much lower than the ^{2}D flow rate. There is also a restriction on the diameters of the two columns, because the volume of the fraction collected from the ^{1}D separation is equal to the injection volume on the ^{2}D column. In a few fortunate cases we are able to focus the analytes at the top of the ^{2}D column. For example, if water-soluble polymers are separated by aqueous SEC and the ^{2}D separation is a reversed-phase (RP) gradient. Using a splitter before the second-dimension column (or before the 10-port switching valve) is unattractive, because much of the sample and large volumes of solvent are wasted and because quantitation may be impaired. Therefore, in most cases, the diameter of the ^{1}D column is much smaller than that of the ^{2}D column. This can be achieved either by using a narrow-bore (≤1 mm) ^{1}D column and a very low ^{1}D flow rate (because of the desired long analysis time) or a wide-bore (up to 20 mm) ^{2}D column and a very high ^{2}D flow rate (because of the desired short analysis time). The latter solution is encountered less often because of the large volumes of (organic) solvents required and because common LC detectors do not allow operation at very high flow rates. Relatively large particles (5 or 10 μm) may be used in the first dimension, while very small particles are attractive for the ^{2}D column. Table 2 provides an example of commonly used parameters in LC × LC of polymers.[20]

Table 2. Example of a Column Configuration and Operating Conditions for LC × SEC of Polymers

Parameter	First dimension	Second dimension
Analysis time (min)	180	1
Column diameter (mm)	1	4.6
Flow rate (μL/min)	20	1000
Particle size (μm)	5	3
Column length (mm)	500	50

In gradient-elution LC, the elution strength of the mobile phase increases during the run. Most complex samples contain diverse analytes and to elute each of these under suitable conditions usually requires the use of gradients. The very low flow rates encountered in the first dimension form a complication for gradient-elution. The dwell time (from the start of the gradient until it starts taking effect at the top of the column) and the column hold-up time may be prohibitive. A liquid-flow splitter may be installed just prior to or just after the injector, but the split ratio may vary with composition (viscosity) and thus with time. Moreover, most of the solvent is wasted and it cannot be easily recycled because of the gradient conditions. A more attractive solution is to run the ^{1}D system at a fairly high flow rate during the periods in which no (relevant) peaks are eluted from the ^{1}D column. Using very fast gradients in the second dimension is feasible but not trivial. In conventional gradient-elution LC, much time is lost for returning to the initial conditions and re-equilibration of the column. One way to overcome is to operate two ^{2}D columns alternately,[14] but this requires an additional gradient-elution solvent-delivery system and it puts high demands on the equality of the two 2D systems.

SEC is the most common separation method for polymers [1]. One of its attractive features is that it is performed isocratically. This implies that LC × SEC (gradient elution in first dimension) requires much simpler instrumentation than SEC × LC (gradient elution in second dimension). In LC × SEC, slow high-resolution gradient elution can be performed in the first dimension, using narrow-bore columns. Gradient-elution LC allows relatively high sample loadings, which is important, because the two successive dilutions in LC × LC are generally detrimental for detection sensitivity.[20] In principle, many more detectors are available for LC × SEC than for SEC × (gradient-elution) LC, including the differential-refractive-index (DRI), light-scattering, and viscometry detectors that are especially useful for polymers.

High-resolution SEC separations, using a number of columns in series (as is common in SEC), can be performed in SEC × LC. Narrow-bore SEC columns would be attractive in this context (see Table 2), but these are not readily available. Short, wide-bore SEC columns packed with very small (ideally sub-2-μm) particles, for use in the second-dimension of an LC × SEC experiment, are not amply available either.[22,23] Thus, there is a general need for improved SEC columns for LC × LC separations of polymers. Table 3 lists the strong and weak

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Table 3. Comparative Advantages and Disadvantages of SEC × LC and LC × SEC

	SEC × (gradient-elution) LC	(gradient-elution) LC × SEC
Strong	High-resolution SEC separations possible in ^{1}D	Slow, high-resolution gradients in first-dimension
	^{1}D SEC may serve as sample-cleanup step, removing fractions with very high or very low M_r	High-resolution narrow-bore LC columns readily available
	In some cases (e.g., aqueous SEC × RPLC) focusing possible on top of ^{2}D column	Naturally limited ^{2}D elution window (between total exclusion and total permeation), reducing the effective ^{2}D analysis time
	Peak compression during gradient elution may enhance sensitivity	Broad choice of (SEC) detectors
		Gradient-elution LC allows high sample loads
		No "breakthrough" in ^{2}D
Weak	Narrow-bore SEC columns not commonly available	Short (wide-bore) SEC columns packed with very small particles not readily available
	^{2}D gradients experimentally complicated	Limited efficiency in ^{2}D SEC
	^{2}D analysis time not naturally limited	Limited detection sensitivity
	Risk of "breakthrough", if ^{2}D injection solvent is strong eluent	Composition of ^{1}D effluent may affect ^{2}D separation[24]
	Limited choice of detectors	

points of SEC × LC and LC × SEC, assuming that gradient elution is applied for the LC separation.

A very important issue in developing LC × LC separations is the compatibility of the ^{1}D effluent with the ^{2}D separation. Phase-system compatibility issues often impose severe restrictions on possible methods. The ^{1}D effluent must be miscible with the ^{2}D eluent and it must not have detrimental effects on the ^{2}D separation. A dramatic example mentioned in Table 3 is "breakthrough".[25,26] Using injection solvents that are stronger eluents than the mobile phase at the time of injection is generally not recommended in LC, because it may result in broadened, distorted peaks. In the case of polymers, this effect is much more serious, due to the occurrence of size-exclusion effects in strong eluents. When combining normal-phase (NP) LC with RPLC in NPLC × RPLC, the typically nonpolar ^{1}D effluent may not mix with the aqueous ^{2}D eluent. When applying hydrophilic-interaction LC (HILIC) in the first dimension, the largely organic ^{1}D effluent (containing, e.g., 80% of acetonitrile) may ill-affect the ^{2}D separation.[27] Similar problems may arise when implementing RPLC × NPLC. The volumes of the fractions collected from the first dimension (equal to the ^{2}D injection volumes) can be varied by adapting the ^{1}D flow rate (possibly in conjunction with changes in the ^{1}D column diameter). Large ^{2}D injection volumes are favorable with respect to analyte detectability, but they tend to aggravate phase-system compatibility issues.

Detection is a known weakness of LC. For example, a sensitive detector that yields a linear response for non-UV-active analytes in gradient-elution LC is often not available. Detection problems are amplified in LC × LC because the analytes are diluted in each stage and, in some cases, because of the high ^{2}D flow rates. Detection is aided if the ^{2}D separation is performed isocratically. In fact, a number of detectors can be used in LC × SEC that cannot be used in one-dimensional gradient-elution LC. Detection sensitivity can be significantly enhanced if the analytes can be focused when (or before) they enter the ^{2}D column.

EXAMPLES OF LC × LC OF POLYMERS

Functionality-Type Distributions. One of the most intriguing methods of separating polymers is LC at critical conditions (or "at the critical conditions of adsorption").[28−30] At these conditions, the polymer backbone is thermodynamically equally at home in the mobile phase and on the stationary phase, so that the length of the polymer chain does not affect the retention. This creates interesting options for separating polymers based on functionality (functional groups or end-groups) or for separating block copolymers.[31−33] If separations of functional polymers can be performed at rigorously critical conditions, the fractions containing a given number of end-groups can be determined. One significant disadvantage is that the critical conditions are difficult to incorporate in robust routine methods. The critical conditions have been reported to depend "critically" on the exact mobile-phase composition, the (condition and history of the) stationary-phase surface, the temperature, and even the pressure.[29,34] Another complication is the conversion of the measured concentrations or weight fractions, data that may be obtained using common LC detectors, to numbers of molecules. The latter constitutes the essential information when characterizing reactive (e.g., cross-linking) polymers. Both disadvantages can be overcome by comprehensive two-dimensional LC.

Figure 2 shows the separation of a sample of end-functionalized poly(methyl methacrylate) by LC × SEC, with near-critical, isocratic LC in the first dimension (horizontal retention axis) and SEC in the second dimension (vertical retention axis). Nonfunctional polymers (no reactive end-groups) are contained in the lob on the left-hand side, monofunctional polymers in the lob in the middle, and difunctional molecules in the area indicated on the right-hand side of the figure.

The LC separation in Figure 2 is seen not to be rigorously critical. If the retention in the first dimension would be independent of the molecular weight (reflected by the position on the vertical axis), the three lobs would be completely vertical. Under the present conditions, a one-dimensional LC chromatogram would not yield a good separation between (high-molecular-weight) nonfunctional polymers and (low-molecular-weight) monofunctional polymers. This situation cannot be remedied by adapting the mobile-phase composition, because "more-critical" conditions for the nonfunctional and monofunctional polymers would result in "less-critical" conditions for the difunctional polymers, the area of which is slanted in the opposite direction. Thus, the LC × SEC separations yields information that cannot be obtained from a one-dimensional experiment. The two-dimensional separation is more robust, because maintaining rigorously critical conditions is not a requirement. The recovery of critical-LC experiments has been questioned.[36,37] It is not clear at this stage whether the observed recoveries can be improved by a more rigorous separation such as shown in Figure 2.

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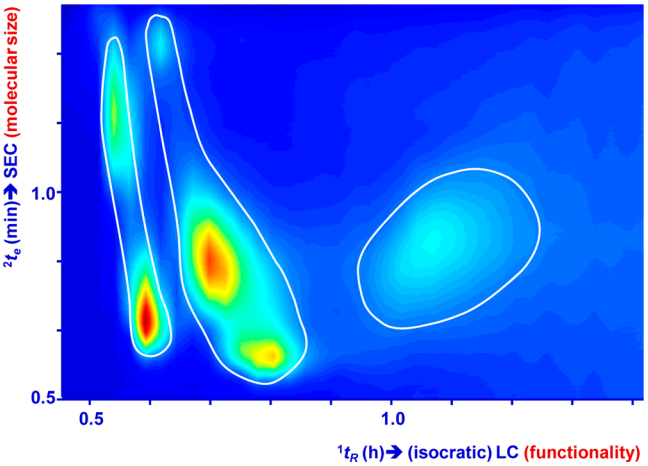

Figure 2. Separation of end-functional PMMA polymers by LC × SEC. Near critical isocratic LC conditions: 150 mm × 1 mm Hypersil silica (ThermoFisher Scientific) column, 48% acetonitrile in dichloromethane, 8 μL/min. SEC conditions: Two 50 mm × 4.6 mm PLGel columns (Agilent) 5-μm 100 Å and 6-μm Oligopore, tetrahydrofuran (THF), 0.9 mL/min. UV detection at 220 nm. Manually added white lines indicate approximate domain borders. Adapted with permission from ref 35. Copyright 2005 Elsevier.

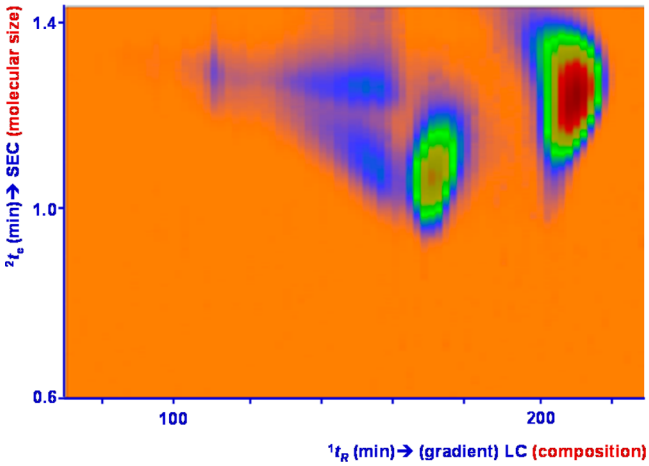

Figure 3. Separation of poly(styrene)-*co*-poly(methyl methacrylate) latex used in coating formulations by LC × SEC with CAD detection. First-dimension LC conditions: 300 mm × 4.6 mm Zorbax Eclipse XDB-C8 column (5-μm particles, 80-Å pores), gradient of 50 to 100% THF in 75:25 H_2O/ACN in 270 min, flow rate 25 μL/min. SEC conditions: 100 mm × 7.5 mm PLGel Mixed C (Agilent), THF, 1.1 mL/min. Colors represent detector response (dark red is highest; orange is background).

After converting the vertical axis to molecular-weight through a conventional SEC calibration, the number of molecules of each type of functionality can in principle be calculated. However, detection is, as always, a crucial factor. The sample of Figure 2 featured UV active end-groups, so that the signals for monofunctional and difunctional polymers could be calibrated using a UV detector. The nonfunctional PMMA showed a low but significant response at the wavelength of 220 nm used for the experiments. A DRI detector often lacks sensitivity, whereas evaporative light-scattering (ELSD) and charged-aerosol (CAD) detectors yield a nonlinear response.

Chemical-Composition Distributions. Arguably the most important type of distribution addressed by LC × LC techniques is the chemical-composition distribution of copolymers.[3−5,38] The ratio of the two (or more) monomeric units in a copolymer has a major effect on many properties. The composition of a polymer may be tuned to obtain desirable properties. Mixtures ("blends") of two different copolymers allow an even greater degree of fine-tuning. The composition of a (statistical) copolymer can be determined using gradient-elution LC (GELC) with suitable calibration.[39,40] However, the molecular weight of the different fractions will not be revealed in such an experiment. Typically, there is a minor effect of molecular weight on retention in GELC for low-molecular-weight polymers (see the tail toward the northwest on the large peak at the top right of Figure 3). Thus, in GELC molecular-weight effects and composition effects may be confounded. In addition, other (minor) fractions with different molecular weights and/or chemical compositions may be present and these may critically affect the resulting properties.

Figure 3 shows the separation of a poly(styrene)-*co*-poly(methyl methacrylate) latex used in coating formulations. The two main fractions (eluting after about 170 and 210 min from the 1D column) are purposefully created in a two-stage emulsion polymerization. The additional (blue) fractions that elute early from the 1D are unexpected ingredients. A one-dimensional SEC separation (projection on the vertical axis) yields a bimodal distribution with little additional information. In a one-dimensional GELC separation, the early eluting fractions are not separated from each other and they may easily be mistaken for a drift in the baseline. Indeed, it is an added advantage of LC × LC that sufficient baseline is available (orange area in Figure 3) to allow unambiguous differentiation between signal and background.

Degree-of-Branching Distributions. A less-common but extremely relevant comprehensive two-dimensional LC separation concerns the characterization of branched polymers. These may be separated in one dimension by a technique called molecular-topology fractionation (MTF[41]). In this technique the mobile phase is forced through channels that are similar in size as the polymer molecules in solution. It is thought that the molecules are deformed during the migration and that linear molecules deform more readily than do branched ones.[42] As a result, linear molecules elute earlier from an MTF column than their branched counterparts. Figure 4a shows the MTF × SEC separation of some polystyrene (PS) "stars". Each PS "arm" has an average molecular weight of 1.3 MDa. Coupling two such arms results in a linear polymer with a molecular weight of 2.6 MDa (signal at around 125 min). Three arms yield a Y-shaped polymer with a molecular weight of 3.9 MDa (signal at around 180 min) and four arms yield an X-shaped polymer with a molecular weight of 5.2 MDa (signal past 300 min). Clearly, very large polymers can be baseline separated based on the presence of one or two long branches. In the case of the Y-shaped polymer, we have one single branch in almost 40 000 units.

The separation of Figure 4a yields simultaneous information on the degree of branching and the molecular weight of the polymer. However, much of the branching information may be obtained from a one-dimensional MTF separation (projection on the horizontal axis). However, this will not be the case for real, randomly branched samples, the characterization of which is of great interest to the polymer industry. An example is shown in Figure 4b for a broadly distributed polystyrene (weight-average molecular weight about 400 kDa). The long-chain-branching of this sample results in a distinct tail toward

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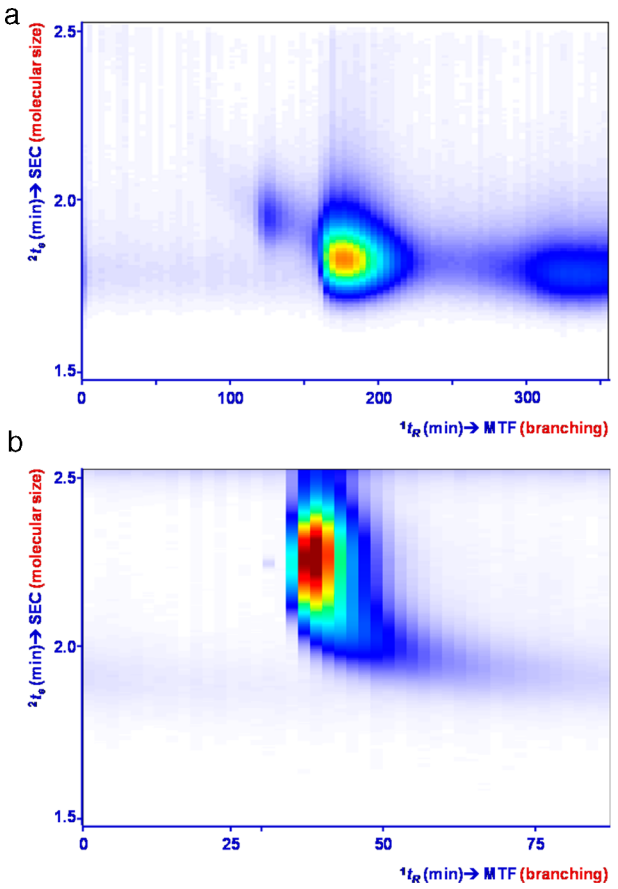

Figure 4. (a) Separation of polystyrene "stars" by MTF × SEC with UV detection. MTF conditions: 150 mm × 4.6 mm i.d. column packed with 0.1 to 1 μm polydisperse silica particles (Admatech, Aichi, Japan), THF at 10 μL/min. SEC conditions: Agilent/Polymer Laboratories 150 mm × 4.6 mm i.d. column packed with 10-μm 10^6 Å PLgel particles, THF at 0.75 mL/min. Adapted with permission from ref 43. Copyright 2008 Elsevier. (b) Separation of polystyrene sample with a broad MWD and a high degree of long-chain branching by MTF × SEC with UV detection. MTF conditions: as in part a, except 20 μL/min; SEC conditions as in part a. Adapted with permission from ref 43. Copyright 2008 Elsevier.

the southeast. MTF × SEC is a highly promising technique for characterizing branched polymers.

SUMMARY AND OUTLOOK

Comprehensive two-dimensional LC (LC × LC) separations of synthetic polymers do not yield remarkable numbers of chromatographic peaks because individual oligomers are not separated within the envelopes displaying molecular-weight, chemical-composition, or other distributions. However, LC × LC separations yield information that cannot be obtained from one-dimensional separations. Without (chromatographic) separations only average data, such as molecular-weight averages, can be obtained on polymers. With one-dimensional separations only the average of one property as a function of a second property, such as the average composition as a function of the molecular size, can be obtained. With two-dimensional separations, comprehensive two-dimensional distributions can be obtained and therewith essential information on the composition of synthetic polymers. In principle, LC × LC allows the use of detectors that cannot be used in one-dimensional separations. For example, (gradient-elution) LC × SEC is compatible with a number of detectors that cannot be used in one-dimensional gradient-elution LC. LC × LC has been successfully implemented for challenging polymer-separation problems. For example, block copolymers have been separated using critical conditions for one block and size-exclusion conditions for the other block in two different orders.[31−33] Polyolefins usually require high temperatures for dissolution and for liquid-phase separations, which has recently resulted in high-temperature LC × LC separations.[44−47]

There are also still some weak points associated with LC × LC separations of synthetic polymers. Although many detectors are potentially compatible with LC × SEC, detection sensitivity, linearity, and the variation of detector response with molecular weight and composition remain significant issues. This complicates the calibration of the signal intensity. Calibration of the retention axis is also complex in many cases. Many methods have been described to calibrate SEC (i.e., convert retention time to molecular weight) *for homopolymers*. The molecular-weight calibration of SEC systems for copolymers is notoriously difficult, and in LC × SEC of copolymers the chemical composition of the polymer analyzed in the second dimension varies from run to run. Fundamentally LC × SEC yields comprehensive two-dimensional chemical-composition × molecular-size distributions or functionality-type × molecular-size distributions. If a concentration detector and a viscometer are used intrinsic-viscosity distributions and, in principle, molecular-weight distributions may be recorded.

The analysis times needed to record the chromatograms of Figures 2, 3, and 4 were not explicitly discussed so far. These are approximately 2, 4, and 6 h, respectively. Although polymer separations are not usually very fast (one-dimensional SEC separations often require 20−60 min), the analysis times required for LC × LC separations is thought to be prohibitively long. This may be improved if contemporary developments in LC can be successfully exploited for polymer separations.[23] In recent years LC has progressed from high-performance to ultrahigh-performance (UHPLC). Pressure limits have increased from 40 MPa (400 bar or 6000 psi) to 100 MPa or more. Particle sizes have been reduced from 3 μm to less than 2 μm. These developments allow performing separations considerably more rapidly (or with greater resolution). We have demonstrated that "shear" degradation on such columns is not a great danger, at least not for carbon-chain polymers such as polystyrene.[48] UHPLC is proliferating very slowly in the field of synthetic polymers.[23] This is because a sufficient selection of dedicated columns (for SEC in particular) is not yet available. Also, UPLC requires narrow columns (to dissipate the heat produced due to the high pressures used) and these in turn pose strong demands on extra-column volumes. This is especially true for (slow-diffusing) polymers. Extra-column volumes occur in the injector and between the injector and the column, although these volumes are usually irrelevant in case of gradient-elution LC. Extra-column volumes also occur between the column and the detector and in the detector itself. The detector-cell volumes that can be tolerated in UHPLC systems are about an order of magnitude smaller than in HPLC systems. Dedicated detectors for polymers, such as light-scattering and viscometric detectors, are optimized for SEC columns with relatively low efficiency and relatively large internal diameters. As a result, they are not compatible with UHPLC systems.

Despite all the obstacles listed above, Uliyanchenko et al. have recently demonstrated UHPLC × UHPLC separations of polymers with a drastic reduction in the analysis time. Figure 5

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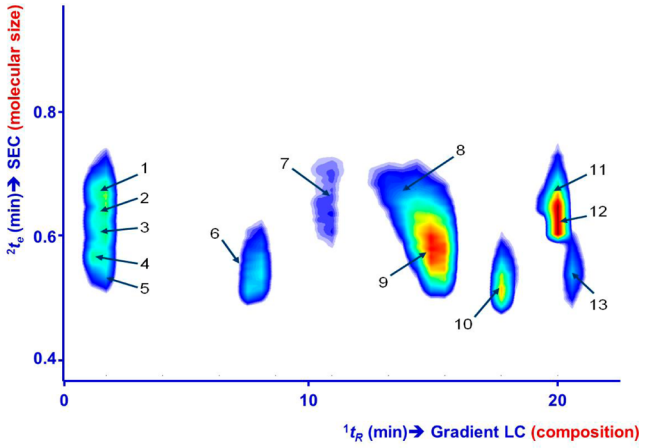

Figure 5. UHPLC × UHPSEC separation of homo- and copolymers of poly(methyl methacrylate), PMMA, and poly(butyl methacrylate), PBMA. 1D: Column Acquity UPLC C18 (Waters), 2.1 mm i.d., total length 250 mm (three columns connected in series). Gradient conditions: 0 to 5 min 15.5% THF in ACN; 5 to 22 min gradient from 15.5 to 80% of THF in ACN. Flow rate 0.2 mL/min. Temperature 25 °C. 2D: Column Acquity UPLC C18, 150 mm × 4.6 mm i.d. Flow rate 2 mL/min. Mobile phase THF. Temperature 30 °C. Peak identification: 1, PMMA/PBMA 100/0, M_r = 15 000; 2, PMMA/PBMA 100/0, M_r = 25 000; 3, PMMA/PBMA 100/0, M_r = 50,000; 4, PMMA/PBMA 100/0, M_r = 65 000; 5, PMMA/PBMA 100/0, M_r = 100 000; 6, PMMA/PBMA 80/20, M_r = 80 000; 7, PMMA/PBMA 65/35, M_r = 20 000; 8, PMMA/PBMA 40/60, M_r = 15 000; 9, PMMA/PBMA 40/60, M_r = 50 000; 10, PMMA/PBMA 20/80, M_r = 110 000; 11, PMMA/PBMA 0/100, M_r = 19 000; 12, PMMA/PBMA 0/100, M_r = 57 000; 13, PMMA/PBMA 0/100, M_r = 100 000. Adapted from ref 49. Copyright 2012 American Chemical Society.

shows an example of a separation of homo- and copolymers of poly(methyl methacrylate), PMMA and poly(butyl methacrylate), PBMA.

CONCLUSION

LC × LC separations are immensely useful for the detailed molecular characterization of polymers, especially for determining comprehensive two-dimensional distributions. They are increasingly applied to characterize complex (mixtures of) polymers. However, there is room for improvement in terms of separation speed and efficiency, detection, and quantitation (calibration). There are plenty of opportunities for greatly improved polymer separations.

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Notes

The authors declare no competing financial interest.

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Petra Aarnoutse studied chemistry in Groningen (The Netherlands) and subsequently worked at several research institutes, specializing in gas chromatography/mass spectrometry. At the University of Amsterdam, she first specialized in spectroscopic techniques, before focussing on polymer characterization by matrix-assisted laser-desorption/ionization mass spectrometry and lately on polymer separations by ultraperformance liquid chromatography.

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