Characterization of complex copolymers by two-dimensional Liquid Chromatography

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Abstract

Complex polymers were characterized by combinations of different chromatographic separation mechanisms: liquid adsorption chromatography (LAC), liquid chromatography under critical conditions (LCCC), and liquid exclusion-adsorption chromatography (LEAC). These techniques were combined off-line and on-line in two-dimensional separations. Fatty acid ethoxylates, fatty esters of polyethylene glycol (PEG) and polysorbates were analyzed by two-dimensional liquid chromatography with normal phase LAC as the first and liquid chromatography at critical conditions (LCCC) or liquid exclusion adsorption chromatography (LEAC) as the second dimension. A full separation of all oligomers to the baseline could be achieved in both dimensions. In two-dimensional separations, the offline approach is compared to comprehensive chromatography, and the scope and limitations of both techniques are discussed.

Keywords: Amphiphilic polymers, surfactants, two-dimensional chromatography, comprehensive chromatography

1. Introduction

In the characterization of polymers there may be different stages of complexity: while linear homopolymers have just a distribution of molar mass (MMD), which can be determined rather easily using simple methods, this is not the case for complex polymers, which may in addition have distributions of functionality and chemical composition, moreover they may differ in their architecture (linear, branched, stars, graft copolymers etc.). The determination of more than one distribution generally requires a two-dimensional separation (and sometimes even multidimensional separations), in which at least one dimension will be liquid chromatography.

A highly promising technique is matrix-assisted laser-desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS). When the first of these instruments appeared on the market, many researchers expected, that this new technique could solve all problems and would sooner or later make chromatography obsolete. Despite its high potential, MALDI-TOF-MS is problematic in the analysis of complex samples, when raw materials are analyzed. In some kinds of samples the same molar mass may be related to several molecules with different composition and architecture. Moreover, mass spectroscopy does not yield any information on architecture (such as

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a discrimination of di- and triblocks). The results obtained with fractions from chromatography are, however, much easier to interpret and typically unambiguous.

Polymers can be separated by liquid chromatography in different modes. Size exclusion chromatography (SEC) separates according to molecular size: elution volumes decrease with increasing molar mass. Liquid adsorption chromatography (LAC) separates according to the number of groups capable of being adsorbed on the surface of the stationary phase, consequently, retention increases exponentially with the number of adsorbing groups in a molecule. A characteristic parameter for these mechanisms is the so-called interaction parameter \( c \) [1]. In SEC, \( c \) is negative, in LAC it is positive. Between these extremes there is a situation, where \( c = 0 \). At a special mobile phase composition and temperature – the so-called critical adsorption point (CAP) – the polymer chain becomes “chromatographic invisible” [2-5], which means, that all chains (regardless their molar mass) elute at the same volume: in the case of non-functional chains at the void volume, while monofunctional chains with adsorbing end groups elute later, but still as narrow peaks. This technique is called liquid chromatography at critical conditions (LCCC). A diblock copolymer with one block (A) at the CAP \((c_A = 0)\) and the other one (B) eluting in the SEC regime \((c_B < 0)\) will elute earlier, i.e. in SEC mode, which allows the determination of the MMD of block B.

At the CAP for A \((c_A = 0)\), A-B diblocks and A-B-A triblocks show the same elution behaviour. A different situation is, however, observed with difunctional chains B-A-B at the CAP for A and adsorbing conditions for the end groups. In this case, retention depends on the size of the critical block! The oligomers elute in SEC order: all polymer chains will elute earlier than a molecule consisting of just two end groups and no repeat units. Obviously, the same situation will also be observed with B-A-B triblocks, where \( c_A = 0 \) and \( c_B > 0 \).

Another situation can also be utilized: If in a diblock A-B the individual interaction parameters have the opposite sign \((c_A > 0 \text{ and } c_B < 0 \text{ or vice versa})\), the individual oligomers elute in SEC order, but far behind the void volume. This technique is called Liquid exclusion adsorption chromatography (LEAC) [6]. It allows a baseline separation of the lower oligomers (up to \( n = 20 \)) of non-ionic surfactants under isocratic conditions.

Using a special software [7], the chromatographic behaviour of even complex polymers may be predicted on the basis of the interaction parameter and several other parameters describing the column (interstitial volume, pore volume, pore diameter). These parameters can be easily determined, as has been described previously [8,9].

These mechanisms can be combined in two-dimensional separations, in which the transfer of fractions from the first to the second dimension can be performed off-line or on-line.

### 1.1 Approaches in two-dimensional chromatography

In the off-line approach fractions from the first dimension are typically collected using a fraction collector. If the mobile phases used in both dimensions are not compatible with each other, the mobile phase from the first dimension is evaporated (e.g. by freeze drying) and subsequently dissolved in the mobile phase used in the second dimension. This procedure is laborious and requires careful handling of the fractions. It reconcentrates the sample components, but unfortunately also any impurity in the solvents. If both mobile phases are compatible, the fractions can be injected directly into the second dimension. The fractions are just filled into autosampler vials, the second dimension can then be operated automatically under appropriate conditions [10]. This procedure is fast and simple, and it avoids the sources of error in sample handling, but suffers from the low sample concentration in some fractions.

The off-line approach has one considerable advantage: fractions can be analyzed by different techniques, and it allows repeated analyses under optimized conditions. Moreover it is possible to analyze the fractions by spectroscopic techniques, among which MALDI-TOF-MS is the most promising one [11].

The on-line approach does not involve any handling of fractions: the eluate from the first dimension is transferred to the second dimension via an appropriate interface (typically a 6-, 8-, or 10-port switching valve) [12]. Obviously, on-line coupling can only be applied, if the mobile phases in both dimensions are compatible. Basically, there are two different approaches: Heart-cutting and comprehensive chromatography.

In heart-cutting, single fractions from the first dimension are directly injected into the second dimension. Alternatively, the fractions may be stored in a short column, where they are completely adsorbed. When the storage column is switched into the second dimension, which runs in a stronger eluent, the fraction is focussed and reconcentrated. Of course, a new injection is required for each peak in the first dimension. This works well, if a sufficient resolution can be achieved in the first dimension.
In comprehensive chromatography, the eluate from an entire chromatogram in the first dimension is collected in aliquots of predefined volumes, which are subsequently injected into the second dimension in an automated way. Obviously, before a fraction from the first dimension is injected in the second dimension, the previous one must be completed analyzed.

1.2. Problems in comprehensive chromatography

The main problem in comprehensive chromatography is the mismatch of flow rates and the sizes of the sample loops and the columns: as an entire chromatogram in the second dimension has to be finished in the time required to fill the sample loop in the first dimension, the first dimension has to be run at a very low flow rate (far from the van Deemter optimum), and the second one at a very high one (which may cause problems with the back pressure). This may be illustrated by the following example: In our case, the Ultimate 3000 pump allows flow rates between 0.1 and 10 ml/min. Hence the flow rate in the first dimension was set to 0.1 ml/min. Under such conditions, a chromatogram on a typical analytical column will take 2 – 3 hours. Using sample loops with 100 or 200 µl, the time to fill the loop equals 1 or 2 min, which is the available time to complete a separation in the second dimension. This means, that one has to use short columns and high flow rates. In practice, monolithic columns should be preferred because of their low back pressure. On such a column, high flow rates are possible, but if an ELSD has to be used, this will cause detection problems. A reasonable sensitivity (which is highly important at the low concentrations in the second dimension) can be achieved a 2 ml/min. This means, that with a loop volume of 200 µl the elution volume of a peak in the second dimension should not exceed 4 ml. If one considers, that the void volume of a column with the dimensions 100x4.6 mm may equal about 1 ml, there is not much space left for a good resolution. On the other hand, injecting 200 µl on such a small column is also quite much, if not too much. With 100 µl loops, however, there is just half the time available for a separation, hence the flow rate in the second dimension must be much higher. This will waste a lot of solvents: a chromatogram of 3 hours will consume 1.2 l of mobile phase, if the flow rate in the second dimension is 4 ml/min.

The next question concerns the mechanism in the second dimension, in which a separation is definitely finished after a given time. This may be SEC or LCCC with the second block in SEC mode, both of which have a rather poor efficiency. In LAC there may be a wrap-over of a fraction to the next interval.

An often neglected fact is also the influence of the mobile phase of the first dimension on retention in the second dimension: if such a large volume of a strong eluent is injected in a solvent stream of a weaker eluent, there may be no retention at all, and the sample will just elute with the solvent peak.

After all, comprehensive chromatography is always a compromise, which may make sense in special cases, but requires very careful optimization. Quite often, other approaches may yield much better results.

A comprehensive separation can also be performed off-line: If the relevant part of the chromatogram is continuously collected using a fraction collector, and the fractions thus obtained are transferred to the autosampler of the second dimension, there is time enough in the second dimension to achieve a good resolution, and the first dimension can also be run at a reasonable flow rate. As we have shown previously [10], a full resolution of all oligomers in a diblock copolymer of ethylene oxide and caprolactone could be achieved in 5 – 6 hours.

2. Experimental

All separations were performed on a gradient system Ultimate 3000, consisting of a pump DGP-3600A, solvent degasser SRD-3600, column thermostat TCC-3000, autosampler WPS-30000SL, all from Dionex (Germerink, Germany), an evaporative light scattering detector PL-ELS 2100 (Polymer Laboratories, Church Stretton, Shropshire, UK). Data acquisition and processing was performed using the software Chromeleon (Dionex, Germerink, Germany). The contour plots in comprehensive chromatography were made using SigmaPlot 11.

In comprehensive LC, the following columns were used:

1st dimension (NP-LAC): Spherisorb 5µ Silica (Waters, Milford, MA, USA): plain silica; 250 × 4.0 mm; particle diameter: 5µm, pore size: 80 Å

2nd dimension (LCCC): Onyx C18 (Phenomenex, Torrance, CA, USA): silica-based monolith with octadecyl groups; 100mmx4.6mm; Mesopore: 130µm; Macropore: 2 µm, Surface Area: 300 m²/g, Carbon Load: 18 %

The solvents (acetone, methanol and water, all HPLC grade) were purchased from Roth (Karlsruhe, Germany).
PEG, fatty esters of PEG and polysorbate samples were purchased from Fluka (Buchs, Switzerland) and Sigma-Aldrich (Vienna, Austria). Several samples of fatty acid ethoxylates were provided by the Institute of Heavy Organic Synthesis (ICSO), Kedzierzyn-Kozle (Poland).

3. Results and discussion

The scope and limitations of different approaches in 2D-LC were evaluated using typical non-ionic surfactants: Fatty acid ethoxylates (or fatty esters of PEG) and polysorbates (commercially available as Tweens). These samples have a different degree of complexity: fatty acid ethoxylates contain PEG, mono- and diesters of the fatty acid, and in the case of technical fatty acids as starting material, the corresponding series with different fatty acids. The fatty acid ethoxylates from ICSO were prepared with pure lauric acid, but with different catalysts, which results in a very different composition [13,14]. The PEG monolaurate is based on technical lauric acid, which contains also myristic, palmitic and stearic acid [15].

Polysorbates are even more complex [16,17]: In the synthesis water is produced by reaction of sorbitol to sorbitan and in the subsequent ring closure to isosorbide. Consequently, the ethoxylation yields the four-armed star of ethoxylated sorbitan and the linear PEG and ethoxylated isosorbide. Hence they contain a hydrophilic core (ethoxylated sorbitan and isosorbide as well as PEG), which is esterified with one or more fatty acids (see Figure 1).

In principle, amphiphilic molecules can be separated according to the length of the hydrophilic part by normal phase LAC (NP-LAC) or according to the hydrophobic part on a reversed phase column.

In Figure 2 typical chromatograms of different fatty acid ethoxylates and PEG 900 are shown, which were obtained by gradient NP-LAC on Spherisorb 5µ Silica in acetone - water with a gradient from 98 to 75 % and 99 to 80 % acetone, respectively. Similar conditions could be applied in the first dimension.

Fig. 1. Possible structures in polysorbates.

Fig. 2. Gradient separation by NP-LAC on Spherisorb 5µ Silica in acetone - water: a) lauric acid ethoxylate (average degree of ethoxylation: n = 9) and PEG 900. Gradient from 98 to 75 % and 99 to 80 % acetone. b) two lauric acid ethoxylates, synthesized with different catalysts (average degree of ethoxylation: n = 6). Gradient from 99 to 80 % acetone.

Figure 3a shows the LCCC separation of the same samples as in Figure 2 and PEG 600 on the Onyx C18 monolith in 90 % acetone – water, which corresponds to the CAP for PEG. At a flow rate of 2 ml/min the last peak
(the diester of ethylene glycol) still appears in time (i.e. before 2 min). As can be seen in Figure 3b, this is not the casewith PEG 400 monolaurate: this samples contains also diesters of higher fatty acids, which will wrap over to the next chromatogram in the second dimension.

Fig. 3.  a) LCCC separation of the same samples as in Figure 2b and PEG 600 on the Onyx C18 monolith in 90 % acetone – water.

b) LCCC separation of the same samples as in Figure 2a and PEG 600 (conditions as above).

Fig. 4. Comprehensive separations (simulation and experiment) of the sample shown in Figure 3a (bottom). First dimension: Spherisorb 5µ Silica in acetone – water, 0.1 ml/min, second dimension: Onyx C18 monolith in 90 % acetone – water, 2.0 ml/min).
Under conditions like these one can try to run a comprehensive separation. Using the parameters mentioned above determined, one may simulate such a separation. As can be seen in Figure 4, the results from simulation and experiment agree very well.

When the contour maps of the two samples shown in Figure 2 are compared (see Figure 4, right and Figure 5, left), it is obvious, that the second sample has a quite different composition. This could, however, be shown much easier by LEAC with a step gradient (right side in Figure 5) [18].

A separation of PEG 400 monolaurate is shown in Figure 6. As can be seen, this sample contains different kinds of diesters and only small amounts of PEG. Again the off-line approach using LCCC – LEAC [15] is superior.

**Comprehensive LC**

**LEAC with step gradient**

Fig. 5. Comparison of comprehensive chromatography (left side, conditions as in Figure 4) and LEAC with a step gradient from 65 to 85 wt-% acetone (right side).

A separation of PEG 400 monolaurate is shown in Figure 6. As can be seen, this sample contains different kinds of diesters and only small amounts of PEG. Again the off-line approach using LCCC – LEAC [15] is superior.
Figure 7 shows a comprehensive separation of Tween 40, which is a highly complex sample. In this case, comprehensive chromatography really makes sense. The individual peaks were identified by spiking with fractions from semipreparative LCCC, which were subsequently analyzed by MALDI-TOF-MS (not shown).

Fig. 6. Separation of PEG 400 monolaurate. Left side: comprehensive LC (conditions as in Figure 4). Right side: Off-line two-dimensional separation: First dimension: LCCC in 90% methanol with focusing of fractions on a FAD column, second dimension: LEAC in acetone water with different compositions for each fraction [15].
An even more impressive picture is obtained with Tween 60 (Figure 8). In this sample even the tri- and tetraesters are visible.

Fig. 7. Comprehensive separation of Tween 40 (conditions as in Figure 4).

An even more impressive picture is obtained with Tween 60 (Figure 8). In this sample even the tri- and tetraesters are visible.
4. Conclusion

In the analysis of complex polymers, different approaches can be applied, which yield different kind of information. Which approach is appropriate, depends on the nature of the sample and the desired information. Comprehensive LC yields a fingerprint of similar samples, but the results are difficult to quantify. On the other hand, for some samples even one-dimensional separations (performed independently) may be sufficient. Off-line 2D-LC has the advantage, that much better resolution can be achieved in the second dimension.

Acknowledgements

We would like to thank Dr. Alexei Gorbunov (Institute for Highly Pure Biopreparations, St. Petersburg, Russia) for providing the simulation software and Prof. Robert Saf (Central Polymer Lab – Structural Analysis (CePOL), Stremayrgasse 16, Graz. Austria) for the MALDI-TOF-MS analysis of raw samples and fractions from LCCC.

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