New Developments in Multidimensional Chromatography of Complex Polymers

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Introduction

Polymers are highly complex multicomponent materials. They are composed of macromolecules varying in chain length, chemical composition, and architecture. By definition, complex polymers are heterogeneous in more than one distributed property (for example, linear copolymers are distributed in molar mass and chemical composition). Depending on the composition of the monomer feed and the polymerization procedure, different types of heterogeneities may become important. For example, in the synthesis of tailor-made polymers, telechelics or macromonomers are frequently used. These oligomers or polymers usually contain functional groups at the polymer chain end. Depending on the preparation procedure, they can have a different number of functional end-groups, i.e., be zero-(e.g., cyclic oligomers) mono-, bifunctional, etc. In addition, polymers can have different architectures, i.e., they can be branched (star- or comb-like).

One very efficient approach for the analysis of the molecular heterogeneity of complex polymers is their chromatographic separation by combining different separation mechanisms. A typical experimental protocol includes the separation of the sample according to composition to yield fractions that are chemically homogeneous. These fractions are transferred to a size-selective separation method and analyzed with respect to molar mass. As a result of this two-dimensional (2D) separation, information on both types of molecular heterogeneity is obtained. So far, 2D chromatography has been applied mostly to polymers that are soluble in organic solvents. There are several problems related to the use of aqueous mobile phases in polymer chromatography. These problems relate to the very polar or ionic character of the polymers and the experimental conditions, including the use of salt-containing eluents. The present paper addresses the different parameters that influence the chromatographic experiments. For a model polymer system resulting from the grafting of methacrylic acid (MAA) onto poly(ethylene glycol) (PEG), i.e., PEG-g-PMAA, it will be shown that different chromatographic techniques including SEC, LC-CC, and 2D chromatography, as well as coupled LC-CC/FT-IR can be used to analyze the molecular complexity of the copolymers.

Summary: Complex polymers are distributed in more than one direction of molecular heterogeneity. In addition to the molar mass distribution, they are frequently distributed with respect to chemical composition, functionality, and molecular heterogeneity. One approach for the analysis of the heterogeneity of complex polymers is their chromatographic separation by combining different separation mechanisms. A typical experimental protocol includes the separation of the sample according to composition to yield fractions that are chemically homogeneous. These fractions are transferred to a size-selective separation method and analyzed with respect to molar mass. As a result of this two-dimensional (2D) separation, information on both types of molecular heterogeneity is obtained. So far, 2D chromatography has been applied mostly to polymers that are soluble in organic solvents. There are several problems related to the use of aqueous mobile phases in polymer chromatography. These problems relate to the very polar or ionic character of the polymers and the experimental conditions, including the use of salt-containing eluents. The present paper addresses the different parameters that influence the chromatographic experiments. For a model polymer system resulting from the grafting of methacrylic acid (MAA) onto poly(ethylene glycol) (PEG), i.e., PEG-g-PMAA, it will be shown that different chromatographic techniques including SEC, LC-CC, and 2D chromatography, as well as coupled LC-CC/FT-IR can be used to analyze the molecular complexity of the copolymers.
molecular heterogeneity is obtained. Another useful approach is the combination of a selective chromatographic technique with a powerful spectroscopic method like NMR or matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry.[1]

2D Chromatography with Aqueous Mobile Phases

There are numerous selective modes for the liquid chromatography (LC) of polymers, including liquid chromatography at the critical point of adsorption (LC-CC)[2–4] and isocratic or gradient HPLC.[5,6] Using these techniques, polymers can be separated selectively with regard to chemical composition or functionality, as has been shown for macromonomers, random and block copolymers, and polymer blends.[6–9] LC-CC and gradient high-performance liquid chromatography (HPLC) are promising first dimensions in a 2D chromatography setup. These can be combined with size exclusion chromatography (SEC) in the second dimension that yields the corresponding molar mass distribution.

So far, 2D chromatography has been applied to polymers that are soluble in organic solvents. Very typically, binary eluents are used in the first dimension, e.g., tetrahydrofuran (THF)-hexane, while in the second dimension THF is the eluent. At present we are not aware of any applications where aqueous mobile phases are used in both dimensions when LC-CC and size exclusion chromatography (SEC) are coupled in the 2D experimental setup.

There are several challenges related to the use of aqueous mobile phases in polymer chromatography. The types of polymers that are to be analyzed using such conditions are water-soluble polar or ionic copolymers. Even the SEC analysis of copolymers analyzed in aqueous mobile phases is not straightforward. In addition to the fact that only a very limited number of stationary phases is available for aqueous SEC, in most cases the experiments cannot be conducted in pure water. Because of the high polarity or the ionic nature
of the polymers, electrolytes or ion-pairing reagents have to be added to the eluent to screen ionic interactions with the stationary phase (for more details see Mori and Barth).\[^{10}\] Salt solutions, on the other hand, can cause severe problems for the SEC detector.

As for HPLC in aqueous mobile phases, there are only a few applications in the literature so far. A number of publications deal with the analysis of polyalkylene oxides, e.g., fatty alcohol ethoxylates.\[^{11–14}\] Two very recent publications describe the analysis of poly(styrene sulfonate) and poly(acrylic acid) in aqueous mobile phases.\[^{15,16}\] On the analysis of water-soluble copolymers by HPLC methods and the determination of the chemical composition distribution of such copolymers, the only publications relate to copolymers of ethylene oxide and propylene oxide\[^{17}\] and partially hydrolyzed poly(vinyl acetate).\[^{18}\]

**Experimental Part**

**Samples**

The graft copolymers were prepared according to the following general procedure: A four-necked flask equipped with a thermometer, a stirrer, and two dropping funnels was charged with 300.00 g of the poly(ethylen glycol) (PEG) and 9.00 g of water. The reaction vessel was heated to 145 °C and purged with nitrogen. Starting simultaneously, the individually calculated amount of methacrylic acid and a solution of 4.50 g tert-butylperoxybenzoate in 10.00 g dipropylene glycol were separately added dropwise with stirring over a period of 2 and 3 h, respectively. Thereafter, the temperature was further maintained at 145 °C for 2 h to complete the reaction. Finally, the reaction mixture was diluted with water, cooled, and the pH 7 was adjusted by adding a 25% aqueous solution of sodium hydroxide. The resulting samples with a 30% (w/w) solid content were diluted with the eluent to a concentration of 3 g·L\(^{-1}\) for one-dimensional measurements and to 30 g·L\(^{-1}\) for coupled methods.

**Chromatographic System**

A Shimadzu LC-10AD-VP HPLC system comprising a pump, an autosampler, and a RI-detector was used. For 2D experiments an ELSD ELS 1000 (Polymer Laboratories, UK) and an additional pump were added. The transfer of the fractions was carried out with an 8-port-2-position switching valve type ET8GW (Valco Instruments Co. Inc.) with two 100-μL sample loops. For data collection and processing, the software package 'WinGPC-Softeware' (Polymer Standards Service GmbH, Mainz, Germany) was used. Molar mass calibration was based on PEG.

**Columns**

The LC-CC experiments were performed on a Knauer Nucleosil RP18-100 (250 mm × 4.6 mm i.d.) HPLC column and the SEC experiments on a PSS Suprema HighSpeed (50 mm × 20 mm i.d.) column. For the 2D measurements, a Macherey–Nagel EC 250/4.6 Nucleosil 100-5 C18 (250 mm × 4.6 mm i.d.) and a PSS Suprema HighSpeed (50 mm × 20 mm i.d.) column were used.

**Mobile Phase**

The mobile phase consisted of mixtures of the following: methanol of HPLC grade (Acros, Belgium), water in ultrapure quality (Simplicity system, Millipore Co.), tris(hydroxymethylamino)methane (TRIS) p.a. (Acros, Belgium), NaCl p.a. (Sigma–Aldrich, USA), Na\(_2\)SO\(_4\) p.a. (Acros, Belgium), and ammonium acetate p.a. (Acros, Belgium).

**Results and Discussion**

**Sample Preparation**

The free-radical grafting was conducted in bulk by reacting the PEG with methacrylic acid and tert-butylperoxybenzoate as initiator. A small amount of water was added to prevent esterification of the PEG and methacrylic acid. According to spectroscopic data, the grafting reaction takes place along the PEG polymer chain and not at the PEG endgroups. Hence, it can be assumed that true graft copolymers are formed.

With the aim to prepare graft copolymers of different molar masses and compositions that could be used as model compounds, a PEG with an average molar mass of 1 500 g·mol\(^{-1}\) was grafted with MAA in different ratios. The MAA/PEG ratios were 15:85, 20:80, 25:75, and 30:70 by wt.-% (i.e., samples 1500-15, 1500-20, 1500-25, 1500-30). These ratios correspond to molar ratios of MAA to ethylene glycol of 8.3:91.7, 11.4:88.6, 14.6:85.4, and 18.0:82.0 mol-%, respectively. When comparing the monomer molar ratios, the samples apparently contain ethylene glycol units in very high concentration. On a molecular level, however, each molecule of PEG is grafted with 3 to 7 molecules of MAA. PEG 1500 has an average degree of polymerization of 34, i.e., the molar ratios of MAA to PEG 1500 are 75:25, 81:19, 85:15, and 88:12. Hence, a complete and uniform graft reaction should yield copolymers with 3 to 7 MAA units per PEG molecule.

**Analysis of the Graft Products by SEC and LC-CC**

The free-radical grafting process of MAA onto PEG is never complete. Considering the fact that during the grafting reaction the polymerization of methacrylic acid homopolymer can take place, very complex reaction products are obtained. Therefore, they consist of the real graft copolymer (PEG-g-PMAA) as well as non-grafted PEG and poly(methacrylic acid) (PMAA). For the characterization and the quantitative determination of the components in the potential mixture of the graft polymer and the by-products, the components must be separated from each other by means of liquid chromatography.
First, the samples were analyzed by aqueous size exclusion chromatography (SEC) to obtain a survey about the molecular heterogeneity. The separation was performed on a PSS Suprema column. The eluent consisted of purified water, 0.08 M TRIS, 0.15 M NaCl, and 0.01 M NaN₃. The pH value was adjusted to 7. The flow rate was 1 mL/min, and the detection was carried out with a refractive index (RI) detector. The RI traces of the different samples are summarized in Figure 1.

All samples exhibit similar SEC patterns: at high elution volumes, a rather sharp and intense elution peak appears that indicates that the samples contain significant amounts of low-molar-mass material. Towards lower elution volumes, peaks or shoulders of variable intensity are obtained that are characteristic for the graft copolymers. A comparison of the elution behavior of the samples with the elution behavior of the initial PEGs indicates that the sharp peaks in the chromatograms are attributable to remaining, non-grafted PEG. The position of the PEG peak is indicated by a vertical line.

The chromatograms show that there is a trend of increasing concentration and molar mass of the copolymer fractions with increasing MAA/PEG ratio. Unfortunately, an exact molar mass and concentration analysis of the samples by SEC cannot be conducted because of the co-elution of the different sample components.

Another technique to separate the reaction products of PEG-g-PMMA and PMAA from residual non-grafted PEG is liquid chromatography at critical conditions (LC-CC) for PEG. Using LC-CC, separation takes place with regard to the chemical composition of the different species and PEG elutes in one chromatographic peak irrespective of molar mass. The reaction products, PEG-g-PMMA and PMAA, have a higher polarity than PEG so should elute under these conditions prior to the PEG peak. Figure 2 summarizes the LC-CC chromatograms of the sample set.

As one can see, baseline separation of the graft products and non-grafted PEG is obtained. For a detailed analysis of the different species, the chromatographic system is coupled with FT-IR spectroscopy.

### Analysis of the Graft Products by LC-CC/FT-IR Spectroscopy

For the transfer of the chromatographic fractions to the FT-IR spectrometer an LC-Transform interface is used. A schematic representation of the experimental setup is shown in Figure 3.

The design concept and the operation of the interface are described in refs. [19–21]. Briefly, the eluate leaving the chromatographic separation system is sprayed onto a rotating Germanium disc through a heated nebulizer nozzle. The mobile phase evaporates and the polymer fractions are deposited as tracks of solid material. After depositing all LC-CC fractions, the Germanium disc is placed into the FT-IR spectrometer.

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Figure 1. SEC chromatograms of the graft products, stationary phase: Suprema linear M, mobile phase: water + 0.08 M TRIS + 0.15 M NaCl + 0.01 M NaN₃, pH 7, detection: RI, samples: 1500-15 (solid), 1500-20 (dotted), 1500-25 (dashed), and 1500-30 (dotted-dashed).

Figure 2. LC-CC chromatograms of the graft products, stationary phase: Knauer Nucleosil RP18-100, mobile phase: methanol/water, 81:19 (w/w), detector: ELSD; samples: 1500-15 (A), 1500-20 (B), 1500-25 (C), and 1500-30 (D).

Figure 3. Schematic representation of the LC-CC/FT-IR coupling using the LC-transform interface.
The FT-IR spectrometer and spectra are taken from all positions of the disc. As a result of the measurements, spectra of all fractions are obtained and are used for identification of the chemical structures, see Figure 4.

A number of selected spectra obtained from sample 1500-25 together with the spectrum of the bulk sample are presented in Figure 5. The spectra correspond to the elution peaks at 1.35 mL (fraction 1), 1.75 mL (fraction 2), and 2.55 mL (fraction 3).

A comparison of the spectra of the different fractions shows that fractions 1 and 2 contain MAA and PEG units. This is not the case for fraction 3 where only the absorption peak for PEG is detected. The low intensity absorption at 1601 cm\(^{-1}\) is probably caused by residual water in the sample. Accordingly, fraction 3 corresponds to residual non-grafted PEG, as has been assumed from the LC-CC chromatogram.

A comparison of the spectra of fractions 1 and 2 reveals that they contain different forms of MAA. While fraction 1 exhibits peaks for the carboxylic (1710 cm\(^{-1}\)) and the carboxylate (1563 cm\(^{-1}\)) forms, fraction 2 does not show a peak for the carboxylic acid. The different chromatographic behavior of the two forms will be a subject of further investigations.

From the LC-CC chromatograms the relative amounts of non-grafted PEG in the samples can be determined. They are summarized in Table 1 and support our previous assumption that the amount of non-grafted PEG decreases with increasing MAA/PEG ratios.

One advantage of the spray deposition technology is that so called “chemigrams” can be drawn from the series of spectra that were obtained for all sample fractions. The chemigrams result from plotting the absorbance at a specific wavenumber or a specific range of wavenumbers as a function of the elution volume of the chromatographic separation. Using this approach the concentration profile of a specific functional group can be visualized. The Gram–Schmidt presentation corresponds to the intensity profile of all absorptions and, therefore, is the total concentration profile.

Figure 6 presents the Gram–Schmidt plot and the chemigrams for sample 1500-15. The distribution of the ethylene glycol units and the total MAA content (carboxylic acid plus carboxylate) are shown as a function of the elution volume. As can be seen very clearly, the first chromatographic fractions contain the highest amounts of MAA units while the last fraction does not contain MAA and is, therefore, attributed to non-grafted PEG. The partial overlap of the non-grafted PEG with the MAA-containing fraction 2 is a subject of further investigations.

### Table 1. Amounts of non-grafted PEG in the samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MAA/PEG</th>
<th>non-grafted PEG</th>
</tr>
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<tbody>
<tr>
<td>1500-15</td>
<td>15/85</td>
<td>68</td>
</tr>
<tr>
<td>1500-20</td>
<td>20/80</td>
<td>60</td>
</tr>
<tr>
<td>1500-25</td>
<td>25/75</td>
<td>55</td>
</tr>
<tr>
<td>1500-30</td>
<td>30/70</td>
<td>44</td>
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</table>
fractions is caused by spreading of the low-viscosity PEG peak on the Germanium disc.

**Analysis of the Graft Products by 2D Chromatography**

While the chemical composition of the graft copolymer samples can be analyzed in detail by LC-CC coupled to FT-IR spectroscopy, the molar masses of the different sample components must be determined by size exclusion chromatography. This can only be done after the LC-CC separation because SEC alone is not capable of separating the non-grafted PEG from the graft products, as has been shown in Figure 1. Preferably, such molar mass analysis has to be conducted separately for each component to obtain full information. An investigation of the chemical heterogeneity in relation to the molar mass distribution of the sample components is done by on-line two-dimensional chromatography, which combines the two methods LC-CC and SEC that separate into diverging directions of molecular heterogeneity.

2D chromatography is conducted by connecting the LC-CC in the first dimension with the SEC in the second dimension. The operation of the system is explained in the experimental part and in refs. [22–25], a schematic presentation of the experimental setup is given in Figure 7.

![Gram-Schmidt plot and chemigrams obtained from the LC-CC/FT-IR analysis of sample 1500-15.](image1)

![Schematic representation of the 2D chromatography setup combining LC-CC and SEC.](image2)

![Contour plots of the 2D LC separations of samples 1500-15 (a) and 1500-30 (b), 1st dimension: LC-CC, 2nd dimension: SEC, detector: ELSD.](image3)
2D chromatography requires a powerful detector for the second dimension. For applications with organic solvents, like tetrahydrofuran, an evaporative light scattering detector (ELSD) can easily be used. This is not possible for aqueous SEC under the previously discussed experimental conditions. Because the eluent contains salt, the spray nozzle of the detector gets blocked. An RI detector cannot be used in 2D LC because the system uses different eluents in both dimensions. Our attempt to solve the detection problem was to use a volatile salt for the SEC eluent in the second dimension. After a number of experiments, ammonium acetate was found to be suitable as a volatile salt. Using water/ammonium acetate as the mobile phase it was found that this composition of samples 1500-15 and 1500-30 as determined by 2D-LC.

Table 2. Composition of samples 1500-15 and 1500-30 as determined by 2D-LC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PEG</th>
<th>Product 1</th>
<th>Product 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>$\bar{M}_w$</td>
<td>Amount</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g · mol⁻¹</td>
<td></td>
</tr>
<tr>
<td>1500-15</td>
<td>68</td>
<td>1200</td>
<td>13</td>
</tr>
<tr>
<td>1500-30</td>
<td>44</td>
<td>1400</td>
<td>6</td>
</tr>
</tbody>
</table>

As compared to the LC-CC and SEC measurements, the 2D experiments yield much more detailed information on the molecular complexity of the samples. Information on the chemical heterogeneity is presented in the ordinate direction of the contour diagram. The molar mass distribution is plotted in the abscissa direction. As can be seen for both reaction products in Figure 8, three different fractions are detected in the contour plots. By comparison with the starting material, one fraction can be assigned to non-grafted PEG. The molar mass analysis of this fraction gives starting material, one fraction can be assigned to non-grafted PEG. The molar mass analysis of this fraction gives a bimodal graft copolymer distribution where Product 2 is the more advanced reaction product with a higher amount of grafted MAA and a higher molar mass. The quantitative composition of the two samples is summarized in Table 2. Samples 1500-15 and 1500-30 are presented as contour plots in Figure 8. The generation of a contour diagram from the individual SEC chromatograms of each fraction has been previously explained by Pasch et al. [6].

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