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Online Coupling of Two-Dimensional Liquid Chromatography and NMR for the Analysis of Complex Polymers

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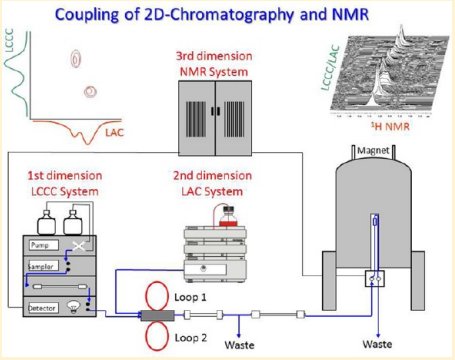
Supporting Information

ABSTRACT: For the first time, comprehensive two-dimensional liquid chromatography (2D-LC) of complex polymers is coupled online to ^{1}H NMR. 2D-LC is used to separate mixtures of poly(ethylene oxide)s with regard to chemical composition and molar mass. The present samples contain polymers with different end groups and chain distributions. In the first LC dimension, liquid chromatography at critical conditions (LCCC) is used for the selective separation according to the end groups. Fractions that are then uniform regarding their end groups are automatically transferred into the second LC dimension which separates the fractions regarding their chain length distributions using liquid adsorption chromatography. The eluate from 2D-LC is directly introduced into the ^{1}H NMR for on-flow analysis. The online coupling of one- and two-dimensional chromatography with ^{1}H NMR detection is demonstrated. The NMR is coupled to both individual separations as well as to the entire two-dimensional separation. As a result of this multidimensional analysis quantitative information is obtained on the types and topology of end groups and the chain length distributions within each functionality fraction.



INTRODUCTION

Comprehensive two-dimensional liquid chromatography (2D-LC) is the most powerful method for the analysis of the molecular heterogeneity of complex polymers. It was developed in the 1990s by Kilz and others[1−3] and since then has been applied to a huge variety of analytical problems. The use of different modes of liquid chromatography facilitates the separation of complex polymer samples selectively with respect to different properties like hydrodynamic volume, molar mass, chemical composition, architecture or functionality. Using these techniques in combination, multidimensional information on different aspects of molecular heterogeneity is obtained.

The experimental aspects and instrumentation of 2D-LC have been reviewed by a number of authors.[4−9] Comprehensive 2D-LC is gaining increasing importance in analytical polymer science for the deformulation of functional homopolymers, random and segmented copolymers, polymer blends and polymers with complex topologies. A number of industrial applications have been reviewed recently by Rittig and Pasch demonstrating convincingly the capabilities of 2D-LC.[10] One very typical example for the useful application of two-dimensional liquid chromatography is the deformulation of graft copolymers.[11,12] Further interesting applications regarding the 2D-LC analysis of epoxy resins,[13,14] aliphatic polyesters,[15,16] polylactides[17,18] and polyamides[20] have been published in the past decade. Significant work has been conducted on the analysis of segmented copolymers, including block and graft copolymers,[19] hydrophilic copolymers,[20−22] and cosmetic copolymers.[23]

The online coupling of liquid chromatography and NMR spectroscopy is known since more than 30 years. Most technical developments, however, were performed during the last 20 years. Albert[24] and Silva Elipe[25] have published an overview of the different developments and applications in LC-NMR.

Two-dimensional chromatography of polymers can be carried out in several ways. In this paper, our main interest is the separation of poly(ethylene oxide)s (PEO) regarding their end groups and chain lengths. Previously, Raust et al.[26] have shown that mixtures of PEOs can be separated by 2D chromatography where liquid chromatography at critical conditions (LCCC) was used as the first dimension to separate the polymers with regard to the end groups and normal phase HPLC was used as the second dimension for separating regarding the molar masses. In addition, HPLC-NMR studies of PEOs have been published previously. The authors demonstrated the separation of technical PEOs by using liquid adsorption chromatography (LAC).[27]

The coupling of LCCC and NMR has been used several times for the characterization of polymers. Kitayama et al. studied poly(ethyl methacrylate)[28] and Hiller et al. analyzed

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polystyrene, poly(methyl methacrylate) and polyisoprene homo- and copolymers.[29−34] Further papers describe the coupling of LCCC and NMR in order to identify the end groups of PEOs.[35,36] SEC-NMR is the most frequently used technique. First studies on SEC-NMR have been published more than 20 years ago. Hatada et al. analyzed homopolymers and copolymers.[37−43] More recent studies used typical chromatographic conditions to analyze copolymers and to determine molar mass distributions.[44−46] Even high-temperature SEC-NMR has been used for the analysis of polyolefin blends and copolymers.[47]

To the best of our knowledge, comprehensive 2D-LC has never been coupled online with ^{1}H NMR. In the present paper, we want to demonstrate the possibility and feasibility of this coupling for polymer analysis. The challenges that had to be addressed are the very low concentrations of the fractions that are obtained by 2D-LC on one hand and the very low sensitivity of NMR as a detector in liquid chromatography on the other hand. Further challenges are related to the fact that in 2D-LC protonated solvents are used that produce very strong NMR signals. These solvent signals must be suppressed efficiently to make sure that all polymer signals are detected.

EXPERIMENTAL SECTION

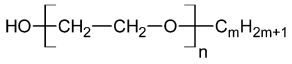
Samples. The PEOs were produced by BASF AG (Ludwigshafen, Germany). A blend of five different poly(ethylene oxide)s was prepared by using equal amounts of each sample of Table 1. The structure of the two types of PEOs is shown in Scheme 1.

Table 1. Poly(ethylene oxide)s Differentiated by End Groups and Chain Lengths (Data Obtained by ^{1}H NMR, LAC-NMR, and SEC-NMR)

		chain lengths		
sample	end group	^{1}H NMR	LAC-NMR	SEC-NMR
1	C_{10}	8.1	8.4	9.7
2	$C_{12,14}$	7.2	8.8	9.5
3	$C_{13,15}$	6.8	7.8	8.1
4	$C_{16,18}$	11.2	11.2	12.0
5	C_9-phenoxy	10.1	11.0	12.9

Scheme 1. Structure of the PEOs Terminated by (a) Alkyloxy Groups (Fatty Alcohol Ethoxylates) or (b) an Alkylphenoxy Group

a)



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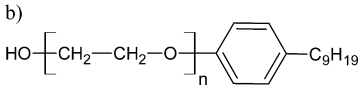

LCCC-NMR (as First Dimension). The LCCC-NMR experiment of the blend of all samples of Table 1 was performed on a 600 MHz spectrometer UnityINOVA equipped with a 60 μL flow probe (triple resonance probe H(C,N) with *z*-gradients). The NMR system was coupled with a Varian ProStar HPLC system consisting of pump, UV detector and loop collection interface.

Critical conditions were achieved by using a Hydro-RP 80A 250 × 4.6 mm column from Phenomenex, and an eluent mixture of methanol/D_2O (80/20 vol %) and 0.05 vol % HCl was used for 20 min, which was linearly changed to 100% methanol within 20 min. The flow rate was 1 mL·min^{-1} and the sample concentration of 20 mg·mL^{-1} for each PEO in the mixture with an injection of 100 μL. The NMR parameters of the onflow experiments were 90° ^{1}H pulse of 3.4 μs, 16 scans per increment, relaxation delay of 1 s, 1 s acquisition time, 12 kb data, and 6 kHz spectral width. The data were processed as 2D contour plots.

LAC-NMR (as Second Dimension). The LAC-NMR system was the same equipment as mentioned above. The chromatographic separation was performed by using a Chromolith Si 100 × 4.6 mm column from Merck. The eluent was a mixture of 2-propanol/D_2O (88/12 vol %) and 0.05 vol % HCl. The flow rate was 0.5 mL·min^{-1}. Each sample of Table 1 was measured separately with a concentration of 10 mg·mL^{-1} and an injection volume of 50 μL. The onflow experiments used 8 scans per increment, 1 s acquisition time, 20 kb data, 10 kHz spectral width).

SEC-NMR (as an Alternative Second Dimension). The SEC-NMR measurements of each sample of Table 1 were executed with a 500 MHz spectrometer DRX (Bruker BioSpin GmbH, Germany) coupled to an Agilent 1100 series HPLC system. A PSS Suprema 100 5 μm 300 × 8 mm column was used. The onflow measurements were performed with a flow probe containing a 60 μL flow cell (90° ^{1}H pulse was 7.7 μs, 16 scans per increment, 0.682 s acquisition time, 8 kb data, 6 kHz spectral width). The data were processed as 2D contour plots (zero filling to 32 kb with a line broadening of lb = 1 Hz). The accuracy of the NMR retention time was ±2 s.

The sample concentration for the SEC-NMR measurements was 20 mg·mL^{-1}. A total of 100 μL was injected. HPLC grade methanol/H_2O (50/50 vol %) was used as mobile phase with a flow rate of 0.3 mL·min^{-1}.

2D-LC-NMR (Coupling of First and Second Dimension with NMR). The coupling of the two-dimensional chromatography and NMR was setup by using a Shimadzu HPLC (Shimadzu Corp., Kyoto, Japan) system comprising a DGU-14A degasser, a FCV-10ALVP solvent mixing chamber, LC-10ADVP pump, and SL 10ACVP auto sampler. The column of the first dimension was a Waters X-Terra (Waters Corp., Milford, MA, USA) RP-18 (2.5 mm average particle size, 127 Å average pore size, 30 × 4.6 mm id). The binary mobile phase was methanol/H_2O (80/20 vol %) with a flow rate of 0.025 as well as 0.03 mL·min^{-1}. Sample fractions from the first dimension were transferred to the second dimension column via an eight-port valve system (type EHC8W, VICI Valco instruments, Houston, TX), which consisted of two loops. Here, 100 and 200 μL loops were tested. The second dimension consisted of a Shimadzu LC-10ATVP pump delivering a flow rate of 2-propanol/H_2O (88:12 vol %) at 1.5 (using 100 μL loops) and 1.0 mL·min^{-1} (using 200 μL loops). A second column, Chromolith C18 from Merck (50 × 4.6 mm id monolithic C18 grafted silica) was added before the Chromolith silica column from Merck (Darmstadt, Germany) (100 × 4.6 mm id monolithic bare silica). A polymer concentration of 20 mg·mL^{-1} per PEO in the blend and 50 μL injected volumes were employed for the analysis. An isocratic mobile phase was used comprising 2-propanol/H_2O (88/12 vol %) and 0.05 vol % HCl with a flow rate of 1 mL·min^{-1}.

The NMR system consisting of a Bruker 400 MHz NMR spectrometer AVANCE and a 60 μL flow probe was directly connected via the output waste line of the second chromatographic dimension to the input of the NMR flow probe. Onflow experiments were performed with 16 scans per FID with 1 s acquisition time, 0.1 s relaxation delay, 90 degree pulse of 4.7 μs, 6000 Hz spectral width, 16 kb data. WET solvent suppression was applied to all solvent signals.[48]

RESULTS AND DISCUSSION

One of the biggest problems for the chromatographic analysis of poly(ethylene oxide)s is the solubility of these polymers. The most common used solvent for chromatography of polymers in particular for SEC is tetrahydrofuran (THF). However, the studied poly(ethylene oxide)s are not soluble in THF. Even water is not appropriate for all samples. Therefore, mixtures of alcohol and water are the only choices for the PEOs. On the other hand, the hyphenation with NMR is more challenging by

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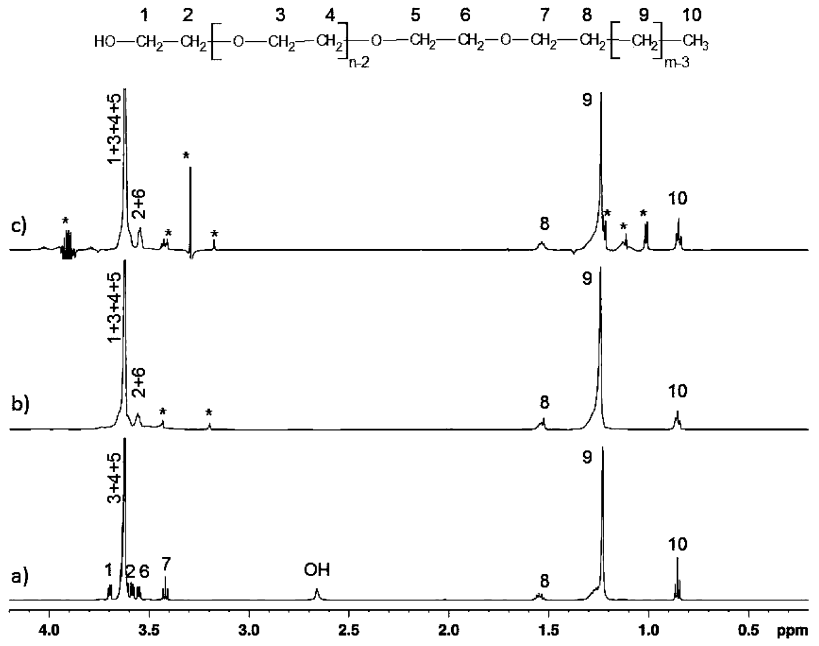

Figure 1. ^{1}H NMR spectra of $C_{16,18}$ alkyl-terminated PEO in (a) $CDCl_3$, (b) methanol/HDO, and (c) methanol/2-propanol/H_2O (assignments are related to the given structure, ∗ indicate remaining solvent signals after WET suppression).

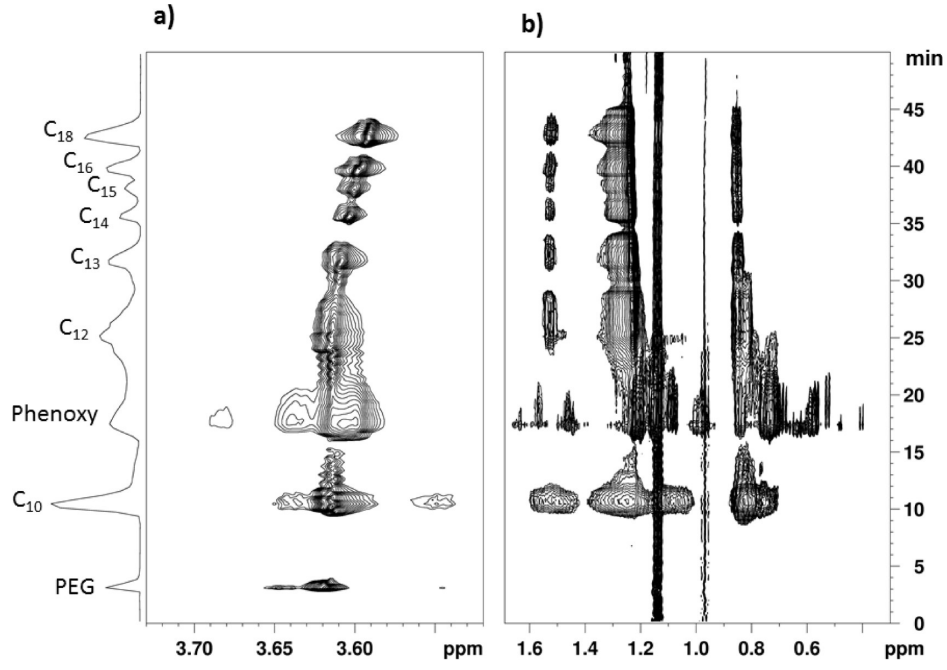

Figure 2. LCCC-NMR of the PEO mixture: (a) OCH_2 region; (b) high field region.

using alcohols. The main reason is the number of solvent signals detected by NMR. Since protonated solvents have to be used each signal of the mobile phase has to be suppressed. This will be one of the major problems for the HPLC-NMR studies of these polymers. In the following the online coupling of the NMR to the individual chromatographic dimensions as well as to the entire two-dimensional chromatography will be demonstrated.

1. LCCC-NMR of the First Chromatographic Dimension. The first chromatographic dimension was used to separate the PEOs regarding to the different end groups. In ref 36, we have shown the LCCC-NMR analysis of PEO mixtures. However, for the present application a different chromatographic phase system was used. Since methanol/D_2O + HCl was used as the mobile phase in the first dimension, only two solvent signals (CH_3 of methanol and OH/HDO) were suppressed (see Figure 1b). In particular, the acid is improving the solvent suppression due to the unique and narrow OH/HDO signal caused by chemical exchange (see also in the last chapter). Figure 1 shows the solvent suppressed spectra of the $C_{16,18}$ alkyl terminated PEO in methanol/D_2O as well as methanol/2-propanol/H_2O in comparison to the spectrum recorded in $CDCl_3$. The spectra of the alkyl phenoxy terminated PEO are shown in Figure S-1 of the Supporting

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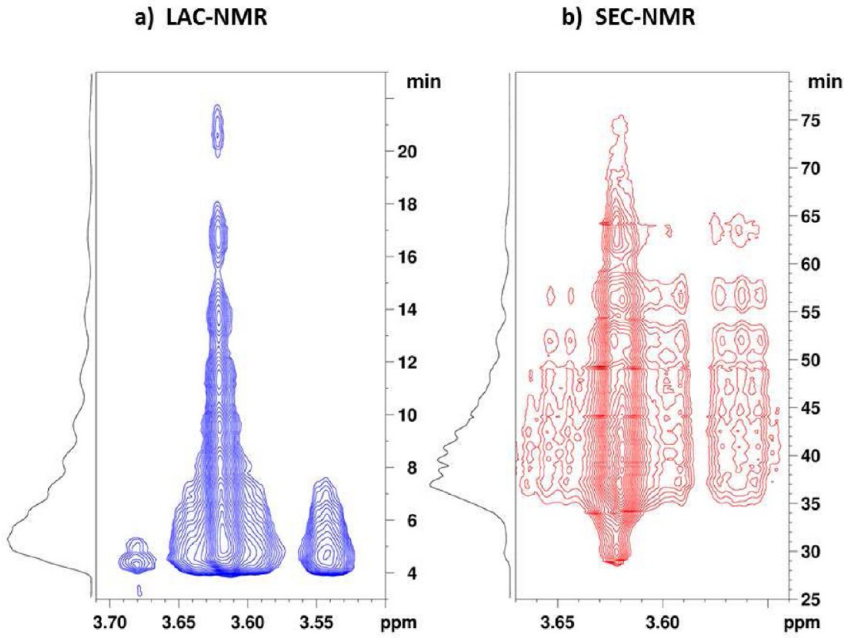

Figure 3. (a) Onflow LAC-NMR and (b) onflow SEC-NMR of the $C_{12,14}$-terminated PEO (sample **2**).

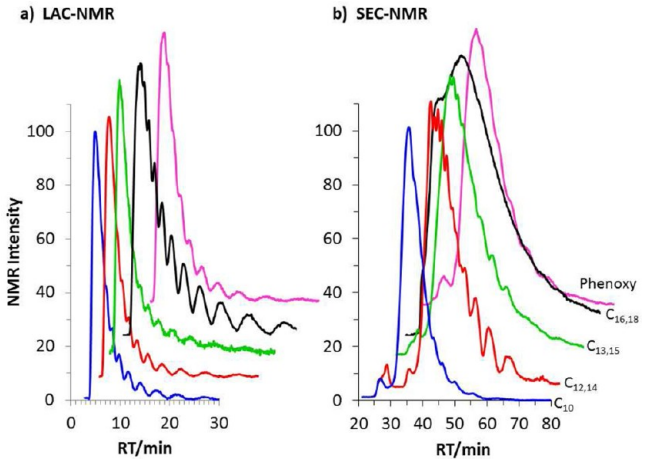

Figure 4. (a) LAC-NMR and (b) SEC-NMR chromatograms of all samples (for the EO main signals).

Information. It is seen in Figure 1b that all protons of the end group can be completely observed and also the main EO units as well as two CH_2 groups. Consequently, the length of the end groups can be calculated as described in ref 36. For the present structure of Figure 1, the calculation would be $m = (2I_8 + I_9 + 2I_{10}/3)/I_8$.

LCCC-NMR is one of the best methods to separate and quantify the end groups of the different compounds. Figure 2 shows the corresponding onflow experiment.

It is clearly visible that nine components could be separated as indicated by the separated EO units (Figure 2a) and their calculated vertical projection which acts as the NMR chromatogram. The end group region (Figure 2b), however, shows only eight separated regions. This finally verifies that the first eluting peak has no alkyl end group and can be identified as poly(ethylene glycol) (PEG). The different end groups are assigned at the NMR chromatogram in Figure 2a. Figure 2b also reveals the topology of the end groups. It is seen from this figure that the C_{13}-, C_{14}-, C_{15}-, C_{16}-, and C_{18}-terminated PEO contain linear end groups indicated by only one CH_3 end group appearing at $\delta = 0.85$ ppm. The other three PEOs, like the C_{10}-, the alkylphenoxy-, and the C_{12}-terminated PEO, are showing many isomers. In this case, the chemical shift region below 0.9 ppm presents several CH_3 groups. This can also be verified by 2D-NMR where the end groups also present CH and several CH_3 groups as an indicator for branching. In particular, the HSQC of sample 5 in Figure S-2 of the Supporting Information shows several CH, CH_2, and CH_3 signals of the aliphatic region. In case of the C_{18}-terminated PEO, the CH_3 resonance can be perfectly used for calculating the length of the alkyl group. The separation according to the end group is also the precondition for determining the chain length. To quantify the length of the PEO chains for each functionality fraction, the separation into these individual fractions must be performed.

2. LAC-NMR or SEC-NMR of the Second Chromatographic Dimension. Normal phase LAC or SEC can be used as the second chromatographic dimension in the 2D-LC setup to separate the different functionality fractions regarding the chain lengths. Both methods use mixtures of alcohols and water as the mobile phase (2-propanol/water for LAC, methanol/water for SEC). As seen in Figure 1c, the main EO as well as signals 2 and 6 can be used for quantification of the chain length by using eq 1 for the alkoxy- and eq 2 for the alkylphenoxy-terminated PEOs. The indices of the integrals are related to the structures of Figures 1 and S-1 (Supporting Information).

$$n = \frac{I_1 + I_3 + I_4 + I_5}{I_2 + I_6} + 1 \quad (1)$$

$$n = \frac{I_1 + I_3 + I_4 + I_5}{2I_2} + 2 \quad (2)$$

LAC- and SEC-NMR measurements were executed for all PEO samples of Table 1. Figure 3 shows the onflow experiments of sample 2 with the plots of the OCH_2 regions (see also Figure S-3 for sample **5** in the Supporting Information). This figure presents a clear separation according

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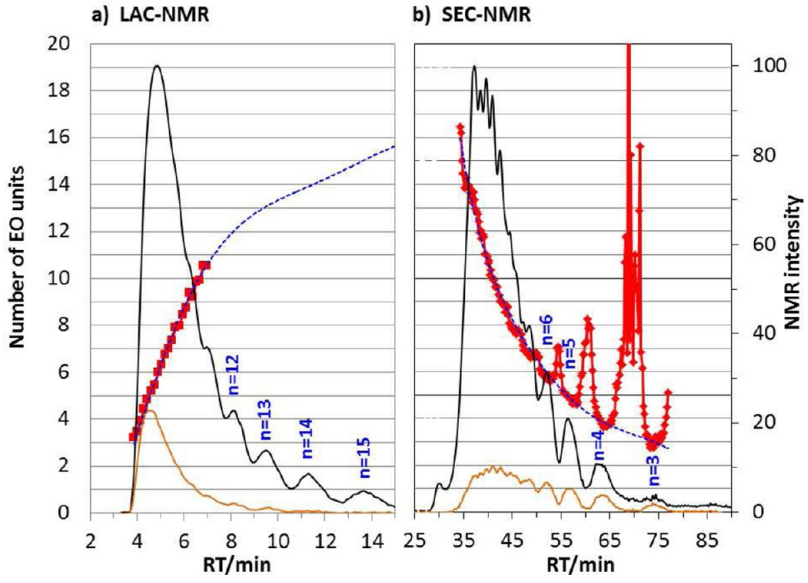

Figure 5. Number of EO units and LAC-NMR and SEC-NMR chromatograms for sample **2** (black solid line = EO main signals, brown solid line = OCH_2 groups 2 and 6, red data points = measured number of EO units, blue dashed line = fitted EO numbers to a polynomial of 4th order for LAC (using all red data points until n = 11 and the points defined by the EO maxima until n = 17) and a polynomial of 5th order for SEC (using all red data points until n = 6 and the points defined by the EO maxima until n = 3) with correlation coefficients of $R^2 > 0.999$).

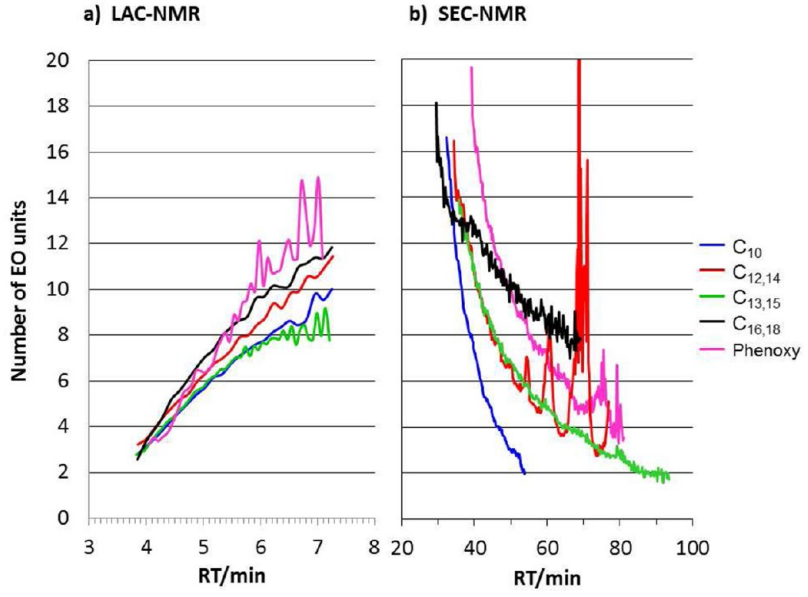

Figure 6. Number of EO units measured with (a) LAC-NMR and (b) SEC-NMR in dependence on the retention time for all PEO samples.

the number of repeat units in the PEO chain. The EO units at 3.62 ppm (signals 1, 3, 4 and 5) and the OCH_2 groups 2 and 6 at 3.55 ppm can be quantified. The NMR chromatogram extracted from this region is shown as the vertical projection. It is also a measure of the quality of the separation.

In order to compare the methods Figure 4 shows the LAC- and SEC-NMR chromatograms of all samples. It can be seen in Figure 4 that some of the PEOs show the typical oligomeric separation profile. The best separation is found for sample **2** which shows more pronounced oligomer peaks for both methods.

Using eqs 1 and 2, the number of EO repeat units can be calculated through the entire elution range. This calculation is shown in Figure 5 together with the NMR chromatograms for sample 2. Figure 5 offers the highest intensity of the LAC-NMR elution for the shortest chains (below n = 11) and for the SEC-NMR for the longest chains (above n = 7). In these cases the number of EO units n could be well calculated. For the other EO numbers stronger scattering is observed due to decreasing signal-to-noise ratio with increasing elution times. However, a very good separation is achieved at later retention and the assignment is given by the maxima with sequential integer numbers. This allows for fitting of the EO number between n = 2 and n = 17 (Figure 5). The strong deviations of n from the dashed line for chains below n = 6 in Figure 5b is caused by the very low intensity of signals 2 and 6 at the minima of the NMR chromatograms where eq 1 delivers large data for n. At the maxima of the chromatogram, however, reliable data are obtained.

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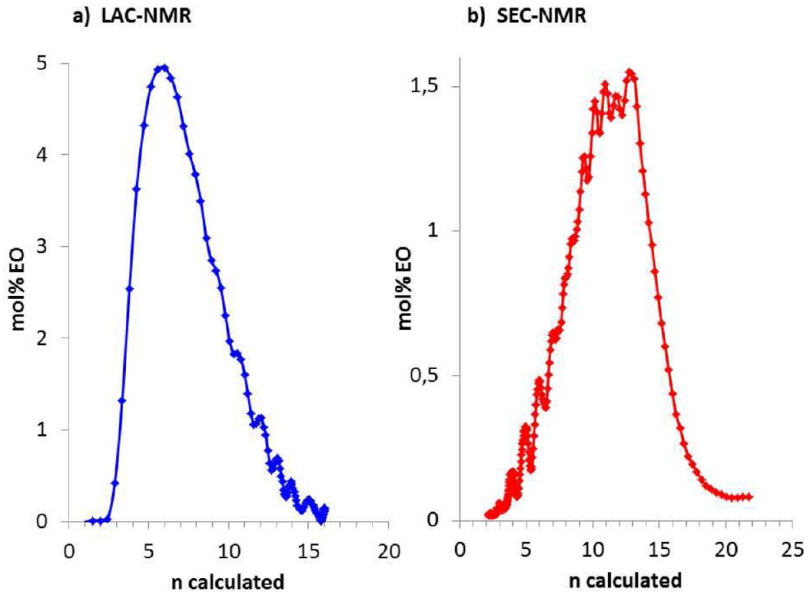

Figure 7. Molar concentration of each EO unit of sample 2 in dependence on the chain length for (a) LAC-NMR and (b) SEC-NMR.

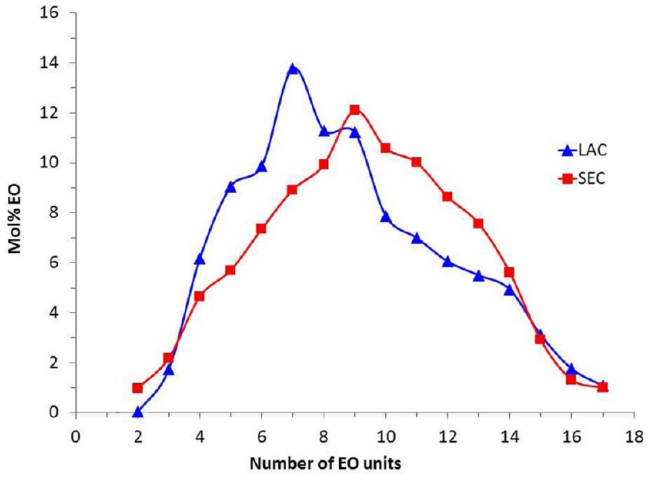

Figure 8. Molar amounts of each chain length of sample **2** by defining the integration regions from Figure 7 for LAC-NMR and SEC-NMR.

Figure 6 shows the number of EO units in dependence of the retention time for all samples. As expected, LAC-NMR separation shows increasing chain lengths with increasing retention time, while SEC-NMR is showing the opposite behavior.

According to Figure 6 the shortest chains consist of 2−3 EO units. This is in contrast to ref 26, which is reporting 5 EO units based on MALDI−ToF measurements. MALDI is also very useful for determining end groups and molar mass distributions. However, NMR might be more reliable due to the fact that even the smallest molecules can be fully detected while in MALDI−ToF very small molecules might be evaporated in the high vacuum of the instrument. Furthermore, NMR can be coupled directly online, is quantitative under flow conditions and can differentiate between isomers. Figure 6a also shows that the elution times of chains with similar numbers of repeat units depend on the type of end group. Only samples 1 and 3 show similar elution behaviors. The $C_{16,18}$ and the alkyl phenoxy terminated PEO tend to elute faster in comparison to the other samples. In case of SEC-NMR, the elution of the PEOs shows a clear trend. The longer the alkoxy group the longer the elution for a given chain length.

On the basis of the fitted chain length dependences, the molar percentage of each EO unit can be continuously calculated. Figure 7 shows a comparison of the LAC- and SEC-NMR for sample 2.

It has to be noted that Figure 7 displays the molar amounts for each measured data point and not for each chain length. Therefore, the curves cannot be compared directly for LAC and SEC. In terms of chain length, for instance, the molar amount for $n = 5$ in Figure 7b is not given by the maximum value (0.33 mol %), but rather by the sum of the data points between the chain lengths of 4.5 and 5.5 (5.7 mol %). Using this approach, LAC and SEC can be compared. Figure 8 shows the calculated concentrations of each chain length by defining regions of ±0.5 around the integers. Moreover, Figure 7 also allows for quantification of the average chain length of the bulk sample. The results for both chromatographic techniques are in good agreement even with off-line ^{1}H NMR measurements (see Table 1).

3. LCCC-LAC-NMR as the 2D-LC-NMR Coupling. The separation of PEOs by two-dimensional chromatography was shown in ref 26. However, the reported chromatographic conditions are not suitable for the online coupling of 2D-LC with NMR. The main reason is the low sensitivity of NMR. To address this problem, the concentration was increased to 20 mg mL^{-1} for each PEO and 200 μL loops were used instead of 100 μL loops. Furthermore, since the NMR is placed after the second LC dimension, it is detecting both methanol/H_2O (from the first dimension) and 2-propanol/H_2O (from the second dimension) with changing compositions in the second dimension. Consequently, the NMR detector experiences a strong LC gradient effect resulting in changing chemical shifts. In addition, different signals appear for the H_2O and OH groups of both alcohols. The latter problem was solved by addition of 0.05 vol % HCl to the second eluent which resulted in only one signal for H_2O and OH groups due to fast chemical exchange. The LC gradient effect was mainly caused by the changing methanol amounts. It could be prevented as follows: a RP column and a switching valve were added before the NP

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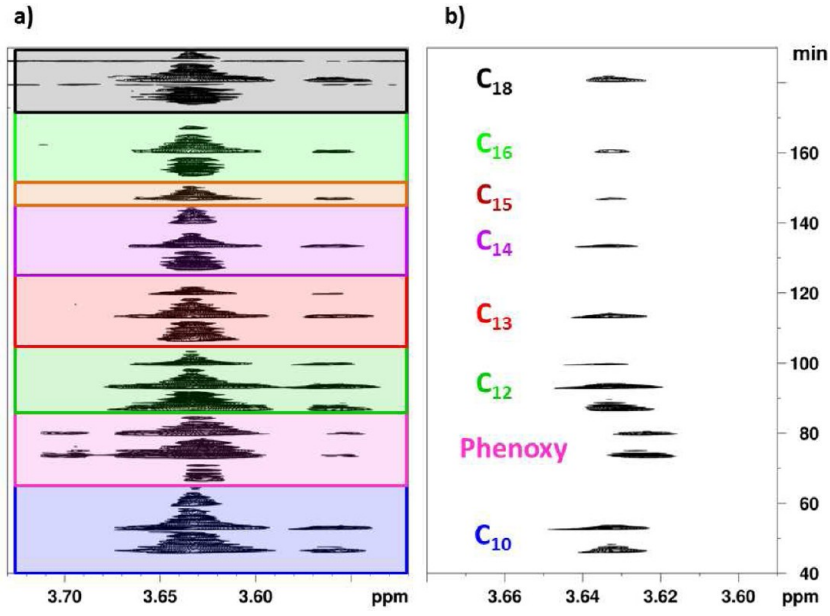

Figure 9. Onflow 2D-LC-NMR experiment of the PEO mixture at (a) higher vertical scale and (b) lower vertical scale of the contour plot (colored regions correspond to the labels in part b.

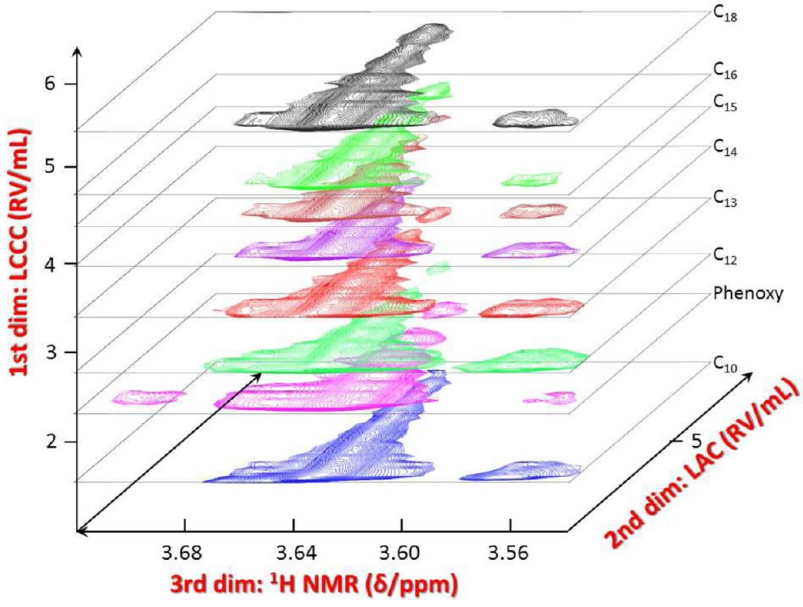

Figure 10. Three-dimensional demonstration of the online 2D-LC-NMR coupling.

column in the second dimension to eliminate most of the methanol. The switch was guiding the flow for 26 s to the waste and then back to the NP column. The duration of the switch to the waste was calibrated according to the volume of the RP column. The start point for the switch depends on the volume of the loop and the flow rates. The major advantage of this approach was a constant chemical shift, an increase of NMR sensitivity and better separation (see Figure S-4). Figure 9 shows the results of the 2D-LC-NMR onflow run of the PEO mixture after including these modifications. In Figure 9a (higher threshold) more details of the separated regions are revealed while Figure 9b (lower threshold) provides a better view for the separated PEOs.

The presentation as 2D contour plots is due to the fact that no three-dimensional processing software exists for this experiment. As the consequence the chemical shift is displayed vs an LC time consisting of both LC dimensions. Therefore, all subsequent separations performed in the first and second dimension are displayed in one plot. Thus, Figure 9a is showing several LAC regions belonging to one PEO. Each of these regions shows a separation according to the second dimension and the sum of these sequential cross sections belonging to one PEO would form the separation according to the first dimension.

Figure 9 can be further processed to build up a three-dimensional picture by extracting the most intense elution peaks from the first dimension and including their profile to the second dimension. Figure 10 is showing the three-dimensional plot. This plot is now containing the elution (given by the retention volumes) of both the LCCC (first dimension) and

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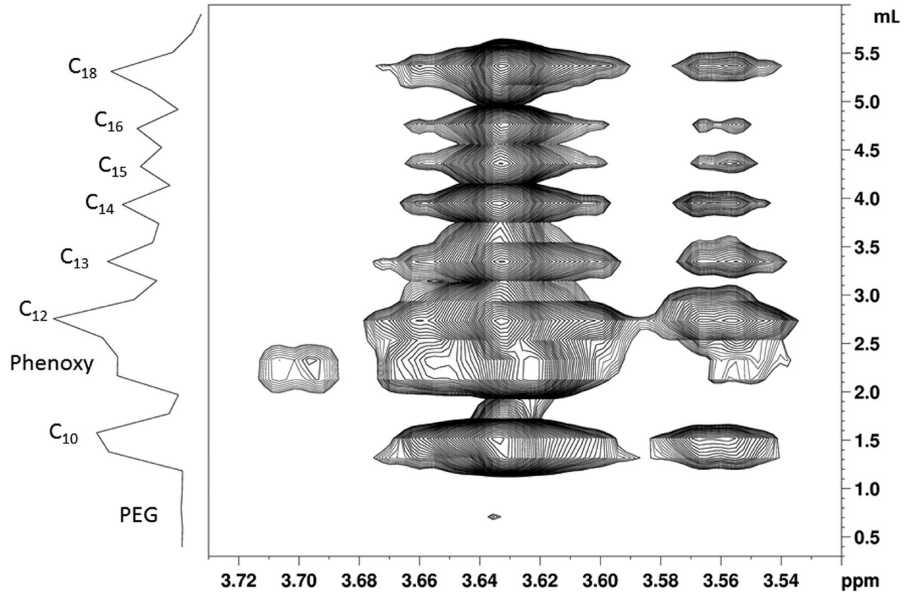

Figure 11. Extracted first dimension of Figure 9 (signals of EO units and CH_2 groups 2 and 6 of all PEOs).

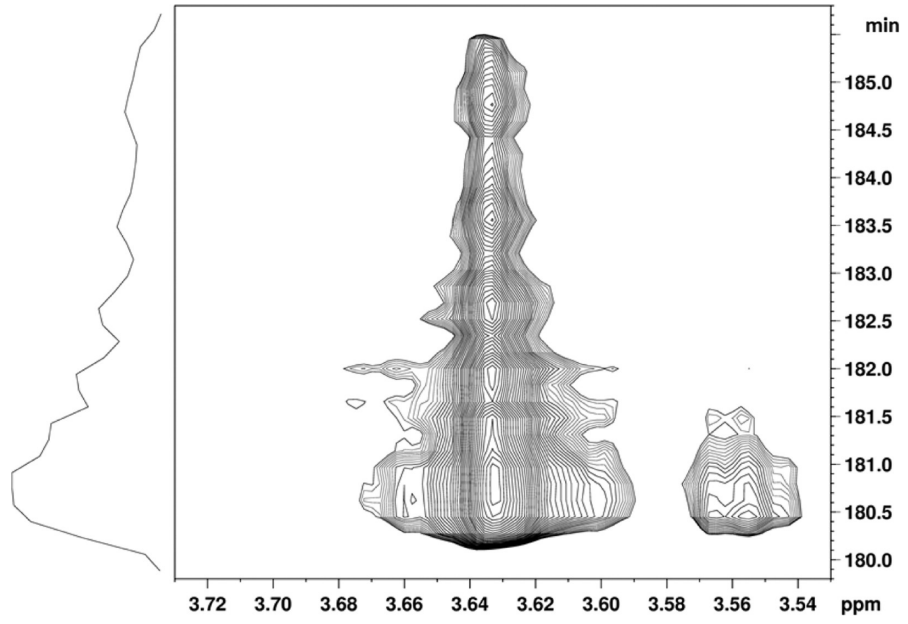

Figure 12. Expanded second dimension of the last eluting cross section of Figure 9a (C_{18}-terminated PEO).

the LAC (second dimension) together with the third dimension given by the NMR chemical shift in ppm.

Figure 10 (or Figure 9) can also be used to extract the first dimension by simply adding the most intense 2D traces. As the result the first dimension (LCCC separation) represented by the main EO signals as well as signals 2 and 6 is shown in Figure 11. The vertical projection of the 2D contours also delivers the NMR chromatogram of the first dimension.

Figures 9 and 10 also deliver all second dimensions by simply expanding the regions. For example, the expansion of the last eluting region corresponds to the C_{18}-terminated PEO and reflects the LAC separation of the second dimension as seen in Figure 12. The vertical projection of this 2D processing is providing the NMR chromatogram of the second dimension.

CONCLUSION

It could be shown that HPLC-NMR is a very powerful tool for the characterization of polymers. By combining NMR with interaction chromatography or size exclusion chromatography direct access to the molecular details of poly(ethylene oxide)s can be achieved. However, it has never been tried to couple two-dimensional chromatography with NMR. It could be shown that this online 2D-LC-NMR coupling can be successfully performed. With this technique it was possible to demonstrate the separation of polymers regarding different end groups as well as regarding the different chain lengths in one experiment. This experiment is not trivial because of the different solvents used for the two different chromatographic dimensions. Limitations of applying this technique are expected for polymers where solubility and chromatographic separation of higher concentrations is a problem. Furthermore, software

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should be developed which is able to extract from this multidimensional experiment the typical two-dimensional hyphenations such as LCCC-LAC, LCCC-NMR, and LAC-NMR.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of alkylphenoxy-terminated PEO, gradient HSQC of sample **5**, onflow LAC-NMR of the alkylphenoxy-terminated PEO, and onflow 2D-LCCC-LAC-NMR traces of the C_{18}- and alkylphenoxy-terminated PEO. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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