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Research Article

Two-dimensional chromatography of complex polymers, 8. Separation of fatty alcohol ethoxylates simultaneously by end group and chain length

A comprehensive two-dimensional liquid chromatography system was developed to precisely describe the molecular heterogeneity of fatty alcohol ethoxylates. The end-group functionality was analyzed by gradient HPLC while ethylene oxide oligomer distributions were characterized by liquid adsorption chromatography. A baseline separation of all functionality fractions irrespective of the ethylene oxide oligomer chain length was achieved on nonpolar X-Terra® C_{18} with a methanol–water gradient, whereas an isocratic flow of isopropanol–water on a polar Chromolith® Si column gave a separation according to the oligomer chain length without interference of the end-group distribution. The combination of these two methods to conduct online two-dimensional liquid chromatography experiments resulted in a comprehensive two-dimensional picture on the molecular heterogeneity of the sample.

Keywords: Fatty alcohol ethoxylate / HPLC / Monolithic stationary phase / Poly(ethylene oxide) / Two-dimensional liquid chromatography
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1 Introduction

Fatty alcohol ethoxylates (FAEs) are one of the most important classes of functional homopolymers. They are also termed alkyl- or aryloxy-terminated polyethylene oxides (PEOs). These oligomers and polymers have a hydrophilic PEO polymer chain and a hydrophobic fatty alcohol or aryl end group. They are industrially used as amphiphilic surfactants, emulsifiers, dispersants, *etc.* FAEs are prepared by anionic polymerization of ethylene oxide (EO) in the presence of mixtures of fatty alcohols having different chain lengths, mainly in the range of C_{10}–C_{18}. Thus, macromolecules with different end groups are formed and the samples exhibit functionality type distributions (FTDs). Like all other synthetic polymers, FAEs exhibit molar mass distributions (MMDs), *i.e.* oligomers with the same end groups have different chain lengths. Finally, the end groups may appear as different isomeric structures. To analyze the structure–property correlations of these complex species, it is most desirable to have a method that separates FAE according to the end groups and the oligomer distributions. Such a method would provide the molecular heterogeneity in terms of FTD × MMD.

It has been demonstrated several times that polyalkylene oxides can be separated efficiently by different methods of interaction chromatography [1, 2]. In particular, the functionality type analysis of PEO can be conducted efficiently by LC at the critical point of adsorption (LC-CC). The separation is typically carried out on nonpolar reversed stationary phases and mobile phases of methanol–water or acetonitrile–water. Under these conditions, the separation is independent of the oligomer chain length and occurs only according to the end groups. An excellent separation of the different functionality fractions, for example, can be achieved on RP-18 columns. Using standard particulate columns (250 × 4.6 mm id) and a flow rate of 1 mL/min, the analysis time is typically around 30–40 min *per* sample [2–4]. The analysis time can be decreased significantly when instead of a particulate column a monolithic column is used

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Abbreviations: EO, ethylene oxide; **FAE,** fatty alcohol ethoxylate; **FTD,** functionality type distribution; **MMD,** mass distribution; **PEO,** polyethylene oxide; **SEC,** size exclusion chromatography

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[5]. For example, by using a Chromolith C_{18} or a Chromolith C_8 column and a flow rate of upto 8 mL/min, the separation time *per* sample can be reduced to about 4 min.

The molar mass separation of the oligomers can be conducted by size exclusion chromatography (SEC). Different from the standard conditions where one sample takes 20–30 min, it has been shown recently that SEC experiments can be conducted in less than 3 min [6–8]. The combination of LC-CC and SEC for the analysis of PEO in a 2-D experimental setup (2D-LC) has been described by Murphy *et al.* [9] and Pasch *et al.* [10]. They showed that the molecular heterogeneity in the coordinates FTD × MMD can be described quantitatively by LC-CC × SEC. In recent years, a number of applications of 2D-LC have been published showing the feasibility of the technique for polyesters, polyacrylates/methacrylates, water-soluble polymers, and polystyrene [2, 11–17].

It is known that the typical FAEs have a degree of polymerization of roughly 5–15. Their molar masses are sufficiently low to obtain an oligomer separation in SEC. Under the conditions of 2D-LC, however, this oligomer separation is not achieved (high flow rates, shorter columns, and lower resolution). Alternatively, the FAE can be separated into oligomers by adsorption chromatography. This has been shown for a number of samples by Trathnigg *et al.* [18, 19], Rissler *et al.* [20],] and others. Several groups have developed models for this kind of separation to describe and predict the interaction strength between the stationary phase and the polymers [21, 22].

The present study describes the combination of two interactive modes of LC in 2D-LC. In the first dimension gradient HPLC is used for the functionality type separation while in the second dimension adsorption chromatography is used for oligomer separation. Using this combination, a simultaneous separation according to the end groups and the degree of oligomerization can be achieved.

2 Materials and methods

2.1 Sample

A representative very complex FAE was prepared by solution blending of five commercial FAE products of BASF AG (Ludwigshafen, Germany). The products were mixed in equal amounts to produce a blend that contained nine different functionality fractions. The types of end groups and the average degrees of polymerization as given by the producer are summarized in Table 1.

Table 1. FAE products that were used to prepare the model blend, *n*: degree of oligomerization.

Product	*n*	End group
C_{10}-oxoalcohol	7	C_{10}
C_{12},C_{14}-oxoalcohol	7	C_{12},C_{14}
Nonylphenyl oxoalcohol	10	Nonylphenyl
C_{13},C_{15}-oxoalcohol	7	C_{13},C_{15}
C_{16},C_{18}-oxoalcohol	6	C_{16},C_{18}

2.2 Gradient HPLC for functionality type separation

The separations according to fatty alcohol end group were carried out on a Shimadzu HPLC (Shimadzu Corp., Kyoto, Japan) system comprising a DGU-14A degasser, an FCV-10ALVP solvent mixing chamber, an LC-10ADVP pump, and an SL 10ACVP auto sampler. The chromatographic column was a Waters X-Terra (Waters Corp., Milford, MA, USA) RP-18 (2.5 μm average particle size, 127 Å average pore size, 30 × 4.6 mm id). A binary mobile phase gradient was run in 4 min starting with methanol/water (80/20% v/v) and going linearly to 100% of methanol. The flow rate was 1 mL/min. The column was equilibrated back to initial conditions at the end of each analysis by flushing the column with 10 mL of methanol/water (80/20% v/v) solution. All experiments were conducted at a column temperature of 25°C. For detection, an evaporative light scattering detector ELSD 1000 (Polymer Laboratories, Church Stretton, England) was used. Polymer solutions were prepared in methanol/water (80/20% v/v, 1 mg/mL) and 20 μL of the solutions were injected for analysis. Data were collected with the software package PSS-WinGPC 7 (Polymer Standards Service, Mainz, Germany).

2.3 Adsorption chromatography for oligomer separation

Separation according to the degree of oligomerization was performed with the same chromatographic equipment. The column in this case was a normal-phase Chromolith Si from Merck (Darmstadt, Germany) (100 × 4.6 mm id monolithic bare silica). An isocratic mobile phase was used comprising isopropanol/water (88/12% v/v) with a flow rate of 1 mL/min. Solutions were prepared with 2 mg of sample dissolved in 1 mL of the mobile phase.

2.4 2-D chromatography

In 2D-LC, the end-group separation is conducted in the first dimension and the oligomer separation in the second dimension. For the first dimension, the analytical conditions were used that were described for the one-dimensional gradient HPLC. Only the flow rate was adjusted to 0.025 mL/min to accommodate the injections into the second dimension. The gradient was then recalculated according to the new flow rate. Gradient and operation times were increased accordingly (Table 2).

Sample fractions from the first dimension were transferred to the second dimension *via* an eight-port valve system (type EHC8W, VICI Valco instruments, Houston,

Table 2. Mobile phase gradient for the first dimension, mobile phase: methanol–water.

Run time (min)	0	160	200	360
H_2O in the eluent (%)	20	0	20	20

TX, USA), attached with two 100 μL loops. The second dimension consisted of a Shimadzu LC-10ATVP pump delivering isopropanol/water (88/12% v/v) at a flow rate of 1.5 mL/min. A second column, Chromolith C_{18} from Merck (100 × 4.6 mm id, C_{18}-grafted monolithic silica) was added before the silica column to separate the polymer from the methanol coming from the first dimension system. The same ELSD was used as in previous analyses.

For 2D-LC, more concentrated polymer solutions were used (20 mg/mL). Twenty-five microliters of these solutions were injected in the first dimension. Data acquisition and processing were performed with PSS-2D-GPC software (Mainz, Germany).

3 Results and discussion

3.1 Functionality type separation

As the FAE end groups were long alkyl or alkylaryl chains, functionality type separation was performed on a RP material. As the nonpolar stationary-phase C_{18}-modified silica gel was used, the stationary phase was expected to show strong enthalpic interactions with the end groups, but only weak interactions with the polar EO chains. The mobile-phase composition at the start of the experiment was set to the critical conditions for polyethylene glycol (methanol/water, 80/20% v/v). These critical conditions were specifically determined for the present system based on several examples of critical conditions for this polymer presented in the review article of Macko and Hunkeler [23].

At LC-CC conditions, PEG which is always present in technical FAEs eluted without interaction from the stationary phase close to the dead volume of the column. The end-functionalized FAE species showed strong interactions and were retained on the stationary phase. An increase of the mobile-phase elution strength (by increasing the methanol content in the mobile phase) was needed to elute the functionality fractions in narrow peaks. A linear increase of the methanol percentage in the mobile phase allowed for a progressive desorption according to the hydrophobicity of the end groups (*i.e.* the fatty alcohol chain length). As the EO part of the molecules did not interact with the stationary phase, the separation was independent of the number of EO units and based only on the end-group chain length.

Figure 1 presents the separation of the model blend that was achieved under these conditions. As can be seen, all functionality fractions are baseline separated from each other eluting in the order of increasing hydrophobicity of the end groups.

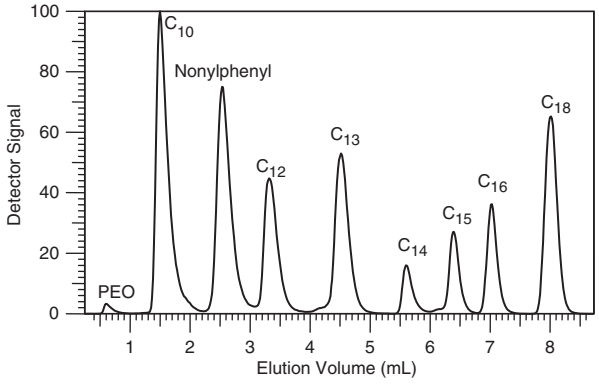

Figure 1. Gradient chromatography of the FAE model blend; stationary phase: X-Terra C_{18}, 30 × 4.6 mm id; mobile phase: linear gradient from methanol–water 80/20 v/v to 100% of methanol in 4 min; flow rate: 1 mL/min; detection: ELSD.

The baseline separation of the different functionality fractions using this method can be accomplished in a very short period of time: only 8.5 min (equal to an elution volume of 8.5 mL) are required for the separation. Such fast separations were earlier described by Pasch *et al.* [5]. Peak assignment has already been confirmed by previous LC-NMR experiments [24]. The order of elution confirms a retention mechanism based on hydrophobic interactions.

Previous experiments, using offline MALDI-TOF-MS measurements, showed that each FAE exhibits an oligomer distribution [24]. No indications of these distributions can be detected with the present gradient experiment. Accordingly, this gradient technique allows for a fast separation according to the end groups of the polymer fractions but without any dependence on the EO oligomer length. Thus, the gradient HPLC method is perfectly orthogonal to molar mass selective methods.

3.2 Chain length separation

In comparison with the FAE end groups, the EO polymer chains are polar. To separate this part of the molecule a polar stationary phase must be selected. Using a normal-phase column (*e.g.* bare silica gel) with an isocratic mobile phase of isopropanol–water, it is possible to separate PEOs according to the EO oligomer length. Such separations were described by Trathnigg *et al.* [25] who showed by the determination of interaction parameters that the EO units adsorb on the stationary phase, whereas nonpolar end groups remain dissolved in the mobile phase and do not interact with the stationary phase. They showed that there was no significant influence of the end groups as long as they were smaller than C_{14}.

The separation according to oligomer lengths was performed isocratically, as it is easier to use as the second dimension for a 2-D chromatographic system. In 2-D

chromatography, fractions from the first dimension are consecutively injected into the second dimension. Normally, 50–100 of such injections are made requiring significant amounts of time. As these injections directly determine the flow rate value (and the total elution time) of the first dimension, each second dimension experiment must be performed as fast as possible. As standard HPLC pumps have difficulties to properly perform a gradient at very low flow rates (limit generally observed at 0.025 mL/min), a fast separation according to the oligomer lengths (in the second dimension) was required to accommodate a reasonable flow rate in the first dimension.

To fulfill the two requirements EO selectivity and fast separation, a short bare silica monolithic column (Chromolith Si, 100 × 4.6 mm id) was selected for the separations. The stationary phase in this case was mesoporic and thus gave lower back pressure than conventional HPLC columns. It allowed for using higher flow rates without being limited by the maximum pressure of the pump. The mobile phase was a mixture of isopropanol and water (88/12% v/v).

Figure 2 shows the oligomer separation obtained for the C_{12},C_{14}-FAE with the fast operating chromatographic system.

The chromatogram obtained for this FAE shows one peak for each oligomer, which confirms that separation occurs without interference of the end groups. As the separation was accomplished by adsorption chromatography, the shortest oligomers eluted first. Indeed, retention was directly dependent on the number of EO repeating units as each of them could be considered as an adsorbing point. The identification of the oligomers was conducted by MALDI-TOF-MS.

When we performed the separation of the complete model blend comprising nine functionality fractions (Fig. 3), we also observed the EO oligomer separation, but a slight peak broadening of the later eluting peaks was seen when compared with Fig. 2. This different behavior was most likely caused by the presence of FAE functionalized with longer end groups (C_{15}, C_{16}, and C_{18}) in addition to the previous ones.

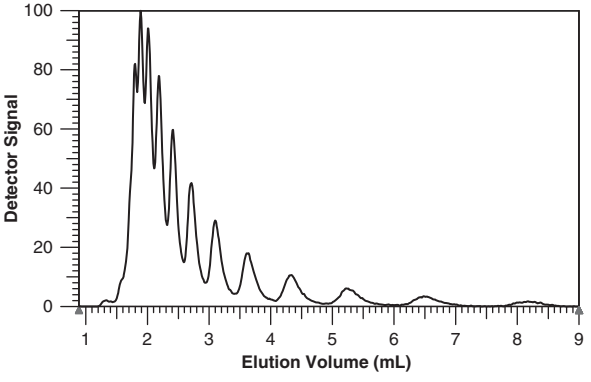

Figure 2. Normal-phase separation of the C_{12},C_{14}-FAE according to the EO content; stationary phase: Chromolith Si, 100 × 4.6 mm id; mobile phase: isopropanol–water 88/12% v/v; flow rate: 1 mL/min; detection: ELSD; samples dissolved in mobile phase.

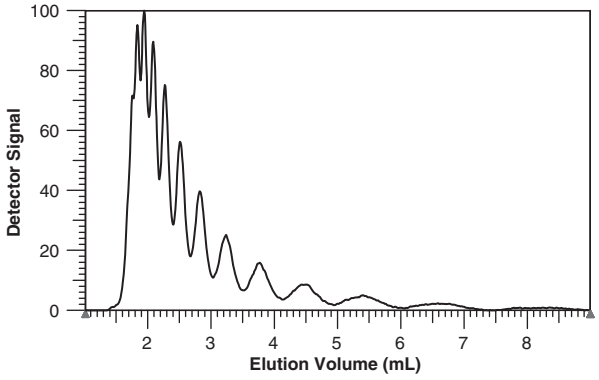

Figure 3. Normal-phase separation of the model FAE blend according to the EO content; stationary phase: Chromolith Si, 100 × 4.6 mm id; mobile phase: isopropanol–water 88/12% v/v; flow rate: 1 mL/min; detection: ELSD; samples dissolved in mobile phase.

This result tends to indicate that the present separation is affected by the end-group length, although an interaction of the end groups with the stationary phase was not expected.

We compared the oligomer elution volumes for the C_{12},C_{14}-FAE with those of the C_{16},C_{18}-FAE. As represented in Fig. 4, a slight difference in elution volumes occurred between the two series: oligomers of the C_{16},C_{18}-FAE always eluted before the corresponding oligomers of the C_{12},C_{14}-FAE. As the end groups were noninteracting species, they should not have any influence on the elution volume of the oligomers whichever end group was coupled to it. But as can be seen in Fig. 4, adsorption of the EO polar units on the stationary-phase surface was indeed influenced by the end-group length.

Different from LC-CC where entropic and enthalpic interactions are balanced against each other, in adsorption chromatography mainly enthalpic interactions are operative. These enthalpic interactions are affected not only by the end group or the polymer chain, but also by the global polarity of the molecules. With an increasing length of the nonpolar end group, the global polarity of the oligomers decreases and hence the polar interactions with the stationary phase also decrease. Thus, oligomers with longer end groups tend to elute earlier. From another perspective, longer nonpolar end groups are more strongly repulsed from the polar silanol stationary-phase surface. Consequently, macromolecules containing such parts tend to be desorbed more rapidly than chains with shorter end groups.

This diagram shows again the importance of a 2-D separation system to properly characterize the present complex model blend. Indeed, molecular characterization of the oligomer mixture would remain incomplete as long as a preliminary separation according to the end-group functionality would not have been performed.

So far the samples were dissolved in the mobile phase isopropanol–water (88/12% v/v). However, while doing 2D-LC experiments, fractions from the first dimension (end-

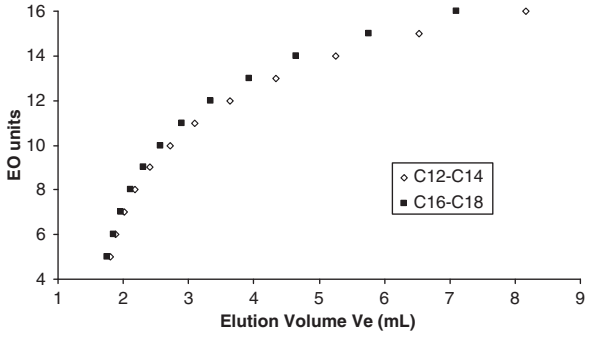

Figure 4. Plot of the oligomer chain lengths *versus* the elution volume for C_{12},C_{14}- and C_{16},C_{18}-FAE; stationary phase: Chromolith Si, 100 × 4.6 mm id; mobile phase: isopropanol–water 88/12% v/v; flow rate: 1 mL/min; detection: ELSD; samples dissolved in mobile phase.

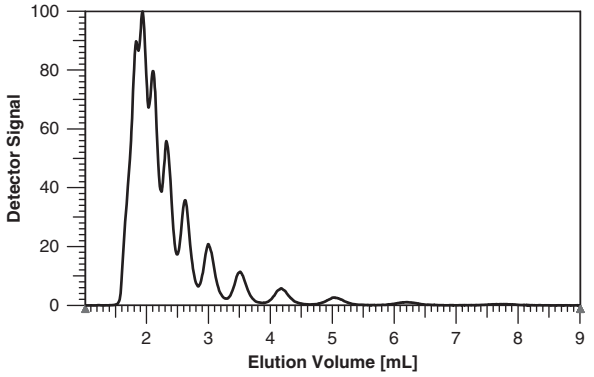

Figure 5. Normal-phase separation of the model FAE blend according to the EO content. Stationary phase: Chromolith Si, 100 × 4.6 mm id; mobile phase: isopropanol–water 88/12% v/v; flow rate: 1 mL/min; detection: ELSD; samples dissolved in methanol–water 80/20% v/v.

group separation) are dissolved in the mobile phase of this system, which is methanol–water. Therefore, we had to check whether the separation in the second dimension is affected by the mobile phase (sample solvent) of the first dimension.

Figure 5 shows the chromatogram obtained from C_{12},C_{14}-FAE dissolved in methanol–water (80/20% v/v) with the fast oligomer separation system (same chromatographic system as used in Fig. 2).

As can be seen in Fig. 5, oligomer separation is considerably less efficient when compared with Fig. 2. The reason was undoubtedly that methanol–water used as solvent was injected into the isopropanol–water mobile phase. Methanol is a good solvent for the oligomers and has a high polarity. Its presence in the silica column did presumably reduce the adsorption strength of the adsorbing system, which resulted in a faster elution of the oligomers. This effect might be even more pronounced when performing 2D-LC as more methanol will be injected in the system at later injections whereas the oligomer concentrations will be lower.

To restore the good oligomer separation, we decided to remove the methanol from the oligomer solutions before they reach the silica column. To achieve this, an RP column (Chromolith C_{18}, 100 × 4.6 mm id) was placed before the silica column. The purpose of this column was to briefly retain the oligomers, whereas methanol should elute without retention.

The comparison of C_{12},C_{14}-FAE and C_{16},C_{18}-FAE on the two columns Chromolith C_{18} and Chromolith Si is shown in Fig. 6.

A comparison of Figs. 4 and 6 reveals that the general relations between elution volume and oligomer length were maintained: retention increases exponentially with the oligomer length. However, with the additional C_{18} column, a delay in the elution was observed: elution started around 3.5 mL instead of 1.8 mL as was found in Fig. 4. This was of course due to the stronger adsorption of the longer end groups on the C_{18} stationary phase.

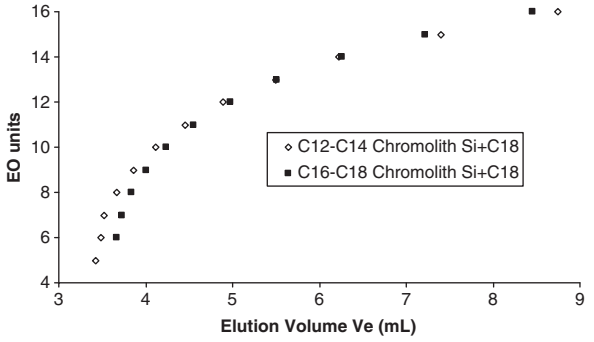

Figure 6. Plot of the oligomer chain lengths *versus* the elution volume for C_{12},C_{14}- and C_{16},C_{18}-FAE; stationary phase: Chromolith C_{18} and Chromolith Si, 100 × 4.6 mm id; mobile phase: isopropanol–water 88/12% v/v; flow rate: 1 mL/min; detection: ELSD; samples dissolved in methanol–water 80/20% v/v.

3.3 2-D chromatography

The ultimate goal of the present method development was the separation of the complex FAE blend simultaneously with regard to the end groups and the oligomer distributions. As was shown in the previous sections, selective end-group separation can be achieved by gradient HPLC while adsorption chromatography is able to provide oligomer separation. The next logical step was now the combination of the two separation protocols in a 2D-LC setup.

The principle of 2-D chromatography is the online analysis of the eluate of a first chromatographic system with a second chromatographic system that is preferably strictly orthogonal to the first. By means of a two-loop switching system, 100 μL fractions of the first dimension eluate are consecutively injected in the second dimension columns. At the time, when one loop is filled, the content of the other loop is separated in the second dimension. It is obvious that the first dimension has to run very slowly, *i.e.* with

0.025 mL/min, whereas the second dimension needs to be set up to a high flow rate, *i.e.* 1.5 mL/min.

As previously mentioned, in the second dimension an isocratic separation method is used, which implies that a reconditioning procedure is not necessary between the two injections.

Figures 7A and B show the results of the 2-D chromatography on the model FAE blend in two different views. The directions of the separations are indicated in the plots as arrows.

The 2-D plots provide a clear idea on the complexity of the model blend. No doubt, the 2-D separation yields selectivities in the different dimensions that are comparable with the 1-D separations. The projection of the plot on the *Y*-axis would give the chromatogram presented in Fig. 1 with the eight baseline separated peaks, which is characteristic of the separation according to end groups. Accordingly, a projection on the *X*-axis would give a chromatogram equivalent to the one presented in Fig. 3. Careful examination of the 2-D plot shows that the series of spots for each functionality fraction represent the corresponding oligomer separation. Thus, in this kind of representation, each spot corresponds exactly to one EO oligomer with a defined end group (*i.e.* one spot for each oligomer). The 2-D plot in Fig. 7B shows the peak distributions even more clearly. The third dimension in this case is the signal intensity detected by the ELSD.

The 2-D plot clearly indicates that the separations in the two dimensions are nearly orthogonal as was expected. Only slight curvatures for the oligomer distributions are obtained in the *X*- and *Y*-axis directions.

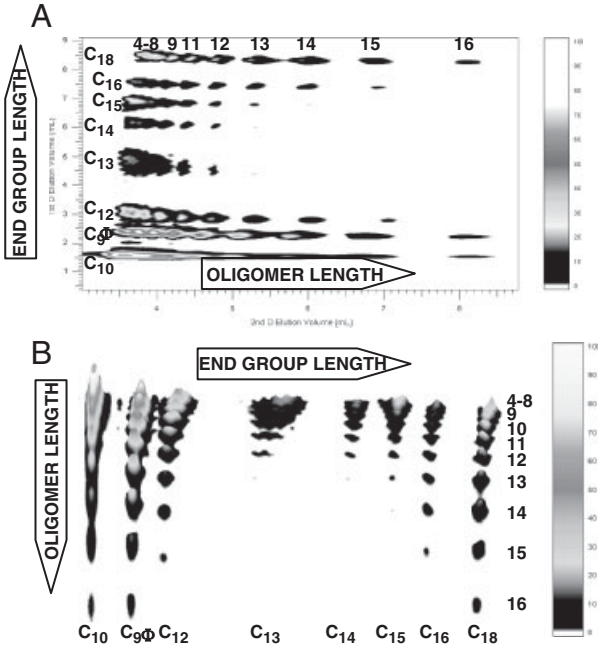

Figure 7. (A) Two-dimensional plot of the FAE model blend (20 mg/mL in methanol–water 80/20); end-group separation (first dimension) along the *Y*-axis and oligomer separation (second dimension) along the *X*-axis; experimental conditions are described in the text. (B) 2-D plot of Fig. 7a after rotation of 90° and inclination of 20°.

As can be seen in both the Figs. 7A and B, the oligomer distributions are very different for the different functionality fractions. For example, the average degree of oligomerization for sample 2 (C_{12},C_{14}-FAE) is 7 (Table 1). Nothing is known about the differences between the C_{12} and the C_{14} fractions. The 2-D plot indicates significant differences between the two fractions. The highest degree of oligomerization that can be detected for the C_{14} fraction is $n = 12$. For the C_{12} fraction, however, oligomers up to $n = 15$ can be detected. A similar behavior is observed for sample 6 where the higher oligomer part of the C_{18} fraction is more pronounced when compared with the C_{16} fraction.

These results show how useful it is to use 2-D chromatography for the detailed analysis of complex polymer mixtures. As was shown, direct coupling of information coming from two different kinds of separations enables us to draw a precise map of polymer heterogeneity in only one experiment which is more pertinent than average measurements on the bulk samples.

With the present experimental conditions, the full duration time for one 2-D analysis was 6 h. Attempts to reduce this time were not successful. In principle, the total analysis time can be reduced by two means, (i) an increase in the volume of the switching loops or (ii) an increase of the flow rate in the second dimension.

An increase in the loop volume to 200 μL led to poor oligomer separation. The amount of methanol injected in the second dimension was twice as much and the Chromolith C_{18} column was unable to separate this solvent from the polymer fractions. This resulted in a dramatic loss of oligomer resolution as has been shown in Fig. 5; single oligomer spots could not be obtained in the 2-D plot.

The increase of the flow rate in the second dimension from 1.5 to 2 mL/min resulted in a very high back pressure. The second limitation for increasing flow rate was the perspective of coupling this 2-D technique with ^{1}H-NMR. A flow rate of 1.5 mL/min was nearly the upper limit to perform on-flow NMR with our equipment.

4 Concluding remarks

Two fast and efficient LC separations were set up to characterize FAE heterogeneity: end group functionality and oligomer length. Coupling both separations in a comprehensive 2-D chromatographic system allowed separation of each kind of chain present in a FAE mixture; each combination of oligomer length and end group was isolated. Specific optimizations were required to maintain separation efficiency of both the chromatographic systems, especially the second dimension – oligomer length separation.

The last step of our analytical development to characterize the FAE is to perform a successful coupling of the 2-D chromatography with an online spectroscopic technique such as ^{1}H-NMR. This hyphenation will enable us to

precisely define the chain structure and the end-group topology of each spot present in the 2-D plot.

The authors have declared no conflict of interest.

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