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# Characterization of ethoxylated fatty alcohols using liquid chromatography with density and refractive index detection II. Quantification in liquid chromatography under critical conditions

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**Abstract**

Ethoxylated fatty alcohols can be characterized by two-dimensional liquid chromatography under critical conditions (LCCC) as the first and size-exclusion chromatography as the second dimension. The effect of preferential solvation in LCCC can be eliminated by the use of two universal detectors in both dimensions, which allows a quantitative determination of all fractions as well as the amount of preferentially adsorbed solvent in LCCC.

## 1. Introduction

In the analysis of ethoxylated fatty alcohols (FAEs), one has to consider that these samples typically consist of different homologous series (depending on the purity of the fatty alcohol used as the starting material), and often also of polyethylene glycols (due to chain transfer to water present in the synthesis).

Hence, a complete characterization of FAEs must provide information on both the distributions of the chain length of the polyoxyethylene and the carbon number of the alkyl group.

This can be achieved using two-dimensional LC, which involves a separation of the homologous series using liquid chromatography under critical conditions (LCCC) [1–4] on a semi-preparative scale as the first dimension, and the analysis of the separated homologous series by size-exclusion chromatography (SEC) [5,6] as the second dimension.

As has been shown in Part I of this series [6], two problems have to be taken into account in the second dimension: (1) the SEC calibrations for the individual homologous series may show considerable differences, hence the individual calibrations should be used for all fractions, and (2) with universal detectors, such as the refractive index (RI) or the density detector, the response factors of FAEs will depend quite strongly on the relative lengths of the alkyl group and the polyether chain [7–9]; this dependence of the response factors on the chemical composition (and molecular mass) can be compensated using different approaches [10]. One of them is also used in this paper.

The molecular mass distribution of oligomers contained in the particular peaks produced under critical conditions can be determined with a good accuracy by the following SEC measurements;

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however, the amount of oligomers within LCCC peaks is uncertain. Since LCCC is typically performed in mixed eluents (such as methanol–water, acetonitrile–water, acetone–water etc.), preferential solvation [11–16] of the oligomers will take place, which leads to vacancy peaks, when universal (bulk property) detectors are used. If the extent of preferential solvation varies with the oligomer composition or molecular mass, the individual peaks will contain different amounts of preferentially adsorbed solvent.

As we have shown previously [16], the extent of preferential solvation of the repeating units and the end groups can be considerably different even in the cases, where this effect is generally neglected. For FAEs, the amount of solvent preferentially adsorbed by each oligomer will strongly depend on both the relative length of the hydrophobic alkyl group and the hydrophilic polyether chain. Hence, considerable errors may arise in the determination of the mass of the fractions.

In this paper, a method is described which allows an accurate determination of the amount of each particular homologous series using LCCC with dual detection in the first dimension. With SEC as the second dimension, a three-dimensional map of FAEs can be obtained.

## 2. Theory

### 2.1. Preferential solvation in LCCC

When a FAE sample is separated according to the length of the alkyl groups by LCCC for polyoxyethylene, each peak will contain a polymer homologous series [with a given end group and an unknown number of ethylene oxide (EO) units] as well as an unknown amount of preferentially adsorbed solvent.

Hence, there are three unknown variables for each peak, namely (1) the amount of the fraction, (2) the composition of the fraction and (3) the amount of preferentially adsorbed solvent.

If preferential solvation can be neglected, the amount and composition of the fraction can be determined from dual detection (density and RI), as is the case in SEC, where single mobile phases are used.

LCCC is, however, typically performed in mixed mobile phases, because critical conditions can seldom be reached in one-component mobile phases.

The determination of all parameters would require a third detection method: this could be UV detection (only for UV-absorbing samples, but not for FAEs or other aliphatic polymers) or evaporative light scattering detection [17]. There is, however, no satisfactory information available about the dependence of its response on composition and molecular mass of oligomers.

If, however, the composition of the fraction is known, one may determine the amount of fraction and preferentially adsorbed solvent with only two detectors. This information can be obtained by analyzing each fraction by SEC with dual detection in the second dimension.

### 2.2. Quantification in two-dimensional LC with dual detection

The area $X$ of a peak eluting in the first dimension results from the mass $m_P$ of polymer containing the mass fractions $w_A$ and $w_B$ of its components A and B, respectively, the mass $m_S$ of preferentially adsorbed solvent, and the corresponding response factors $f_A$, $f_B$ and $f_S$:

$$X = m_P(w_A f_A + w_B f_B) + m_S f_S \quad (1)$$

It must be mentioned that these response factors are the true ones, which are obtained by injecting the samples on the bypass. On the column the zone of "dialyzed solvent" [11] would be separated from the sample peak, thus yielding the apparent response factors [16].

If the response factors $f_A$ and $f_B$ and the mass fractions $w_A$ and $w_B$ are known, one may calculate the average response factor $f_{av}$ of the polymer using

$$f_{av} = w_A f_A + w_B f_B \quad (2)$$

[In the case of FAEs, which consist of the end groups R–and–OH and a polyoxyethylene (PEO) chain without end groups, $f_A$ is the

response factor of the fatty alcohol ROH, and $f_B$ the response factor of high-molecular-mass PEO.]

Hence one may write

$$X = m_P f_{av} + m_S f_S \quad (3)$$

The mass of preferentially adsorbed solvent is given by

$$m_S = \frac{X - m_P f_{av}}{f_S} \quad (4)$$

As the same mass of preferentially adsorbed solvent must appear in both detectors, one may write

$$\frac{X_D - m_P f_{av,D}}{f_{S,D}} = \frac{X_R - m_P f_{av,R}}{f_{S,R}} \quad (5)$$

wherein the indices D and R denote the peak areas and response factors in density and RI detection, respectively. A simple rearrangement of Eq. 5 yields

$$m_P = \frac{X_D f_{S,R} - X_R f_{S,D}}{f_{av,D} f_{S,R} - f_{av,R} f_{S,D}} \quad (6)$$

from which the amount of polymer is easily obtained.

The amount of preferentially adsorbed solvent can be determined using

$$m_S = \frac{X_D f_{av,R} - X_R f_{av,D}}{f_{av,R} f_{S,D} - f_{av,D} f_{S,R}} \quad (7)$$

## 3. Experimental

The investigations were performed using the density detection system DDS70 (commercially available from A. Paar, Graz, Austria), which has been developed in our group. This instrument has been described in full detail in previous communications [18–20]. In SEC measurements it was combined with a Sicon LCD 201 RI detector, in LCCC with a Bischoff 8110 RI detector.

Each system was connected to a MS-DOS computer via the serial port. Data acquisition and processing was performed using the software package CHROMA [20], which has been developed for the DDS70.

In LCCC, two JASCO 880 PU pumps were used, which were equipped with Rheodyne 7125 injection valves with 50- and a 500-$\mu$l loops, respectively.

Reversed-phase LC was performed in methanol and methanol–water mixtures (from Merck, HPLC grade) on two analytical columns and a semi-preparative column filled with Spherisorb from PhaseSep (ODS2 3 $\mu$m, 100 × 4.6 mm; ODS2 5 $\mu$m, 250 × 4.6 mm; and ODS2 5 $\mu$m, 250 × 10 mm, respectively). The flow-rate was 0.5 ml/min in the analytical measurements and 2.0 ml/min in semi-preparative LCCC. An Advantec 2120 fraction collector was used in the semi-preparative separations.

SEC measurements were performed in chloroform (HPLC grade, Rathburn) at a constant flow-rate of 1.0 ml/min, which was maintained by a Gynkotek 300C HPLC pump. Samples were injected using a VICI injection valve equipped with a 100-$\mu$l loop, the concentration range was 4–8 g/l. A column set of four Phenogel columns, (2 of 500 Å + 2 of 100 Å, 30 cm each), was used for all separations.

The SEC calibrations were obtained using pure oligomers of EO (from Fluka) and SEC standards from Polymer Labs.

Samples were dissolved in the mobile phase, which was taken from the solvent reservoir using a PTFE tubing connected to a Omnifit valve. In order to minimize evaporation, the solvent bottle was sealed with a PTFE tape. Before samples were injected, the syringe was stored in a flask filled the mobile phase, and rinsed several times with the sample (in order to minimize adsorption effects).

The alkanols, polyoxyethylenes, and FAE (Brij) samples were purchased from Fluka and used without further purification.

Pure homologous series were prepared by anionic ethoxylation [21] of pure 1-alkanols using standard procedures. A monodisperse oligomer was synthesized by a modified Williamson synthesis [22–24] from 1-octylbromide and tetraethylene glycol.

## 4. Results and discussion

In order to evaluate the performance of this approach, we prepared several pure homologous series by ethoxylation of pure 1-alkanols and a monodisperse oligomer from 1-octylbromide and tetraethylene glycol.

These samples were analyzed by the two-dimensional LC with coupled density and RI detection.

First of all, the critical conditions for polyethylene glycol (PEG) had to be found. As can be seen from Fig. 1, all PEGs eluted at the same elution volume from an ODS2 column in methanol–water (80:20) as a mobile phase.

When samples were injected on the column, they eluted as a narrow peak, and a vacancy peak appeared, the area of which should correspond to the amount of preferentially adsorbed water. As the next step, we determined the true response factors of water, several 1-alkanols and PEG 6000 by injecting them on the bypass. The results thus obtained are shown in Table 1. Using Eqs. 6 and 7, we calculated the amounts of sample and water in each peak. The results thus obtained are given in Table 2.

As can be seen, the calculated sample masses agree very well with the injected sample size, and so do the amounts of water, whether determined from density or RI detection alone or from dual detection using Eq. 7.

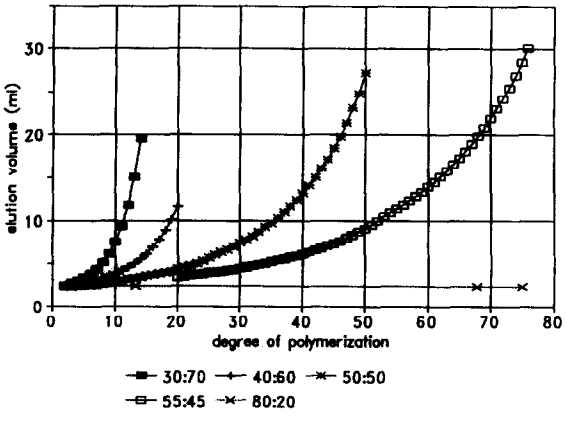


Fig. 1. Elution volumes of polyethylene glycols on ODS2 in methanol–water mixtures as a function of the degree of polymerization.

Table 1
True response factors of water, 1-alkanols and PEGs in density and RI detection, as obtained by injection on bypass

| Sample | $f$ (density) | $f$ (RI) |
|---|---|---|
| Water | 18.44 | 17.32 |
| 1-Octanol | −7.18 | 63.64 |
| 1-Dodecanol | −6.58 | 70.98 |
| 1-Tetradecanol | −6.51 | 73.76 |
| PEG 6000 | 18.09 | 79.05 |

It should be mentioned that the determination of adsorbed water via the vacancy peak is less reliable than the determination using Eq. 7, because the system peak may also contain moisture from the air or may be influenced by adsorption in the syringe.

In Fig. 2, a chromatogram of monodisperse octyltetraethyleneglycol, as obtained by LCCC, is shown. Obviously preferential solvation occurs, as can be seen from the system peak. The small negative peak in front of the sample peak may be explained by traces of a lower oligomer (di- or trimer).

Fig. 3 shows a chromatogram of an ethoxylated 1-octanol, which contained an average of 5 EO units (determined from the EO uptake). This sample contains also a small amount of PEGs, as can be seen from the peak behind the system peak.

It is remarkable, that the peak of unreacted octanol is separated from the polymer homologous series. This could be explained by the existence of residual silanol groups on the column packing. A systematic study shall show, whether different types of octadecyl columns (silica- or polymer-based) behave in the same way. Moreover the long-term stability of the column packing will be investigated.

Fig. 4 shows a chromatogram of a sample with an average of 10 EO units, in which the amount of PEG is considerably larger, and the octanol peak has disappeared. This is quite reasonable, because with increasing conversion octanol should be consumed.

Table 2
Quantification in LCCC (ODS 2; methanol–water, 80:20)

| Sample | Sample size ($\mu$g) | Sample from Eq. 6 | Water ($\mu$g) vacancy peak | | Water ($\mu$g) from Eq. 7 |
|---|---|---|---|---|---|
| | | | Density | RI | |
| PEG 6000 | 197.0 | 193.3 | 82.0 | 79.0 | 78.2 |
| 1-Octanol | 457.0 | 458.3 | 90.8 | 86.5 | 72.9 |

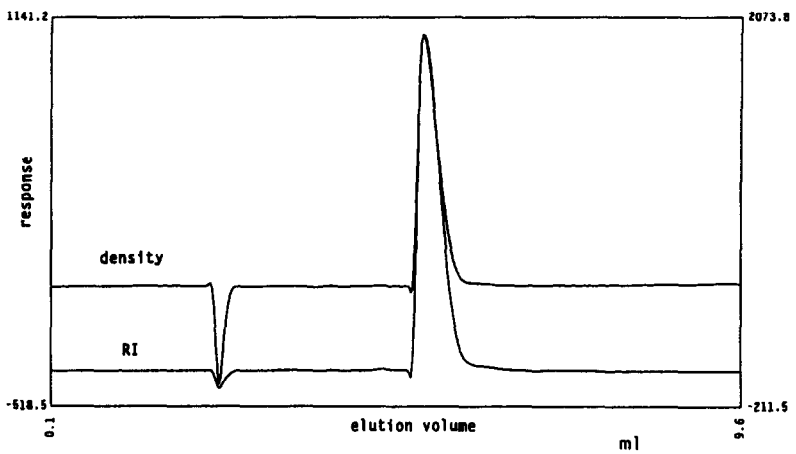


Fig. 2. Chromatogram of the monooctyltetraethylene glycol on ODS2 in methanol–water (80:20, w/w).

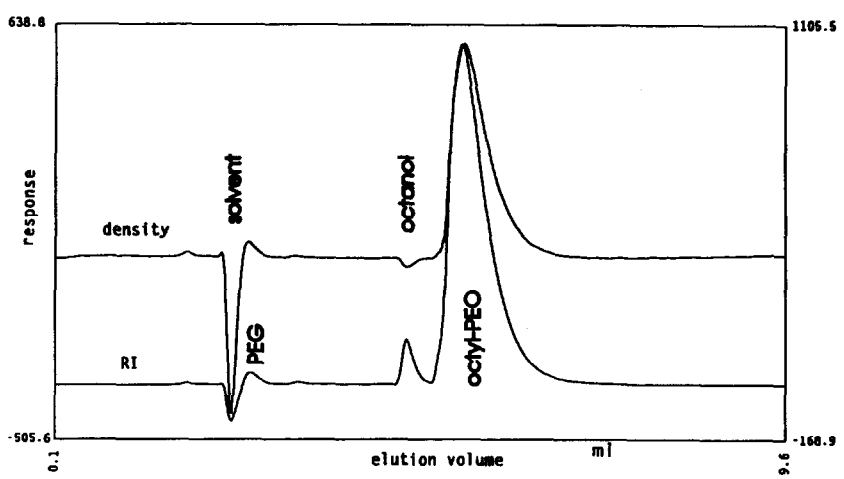


Fig. 3. Chromatogram of the ethoxylated 1-octanol with an average of 5 EO units, as obtained on ODS2 in methanol–water (80:20, w/w).

The fractions from these chromatograms were analyzed by SEC with density and RI detection, as has already been described in Part I of this series [6].

With the mass fraction of the ethylene oxide chain thus obtained we calculated the mass of polymer present in each peak in LCCC, as can be seen from Table 3.

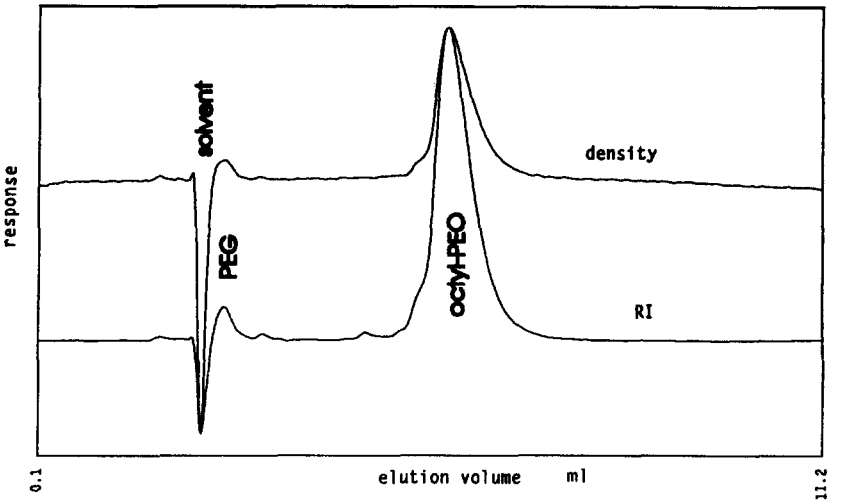


Fig. 4. Chromatogram of the ethoxylated 1-octanol with an average of 10 EO units, as obtained on ODS2 in methanol–water (80:20, w/w).

As can be seeen, the sum of the calculated masses agrees very well with the injected sample size.

## 5. Conclusions

Two-dimensional chromatography with LCCC as the first dimension and with SEC as the second dimension provide an excellent tool for the characterization of polymers. A quantitatively correct three-dimensional map requires, however, an accurate determination of the amount of each fraction in the first dimension. This can be achieved by using a combination of density and RI detector in both dimensions. The three important parameters for each peak in LCCC (the mass of the polymer fraction, its composition and the amount of preferentially adsorbed solvent) can be determined from the corresponding peak areas from both detectors and the average mass fractions of the components (from SEC with dual detection).

Table 3
Masses of the fractions obtained from LCCC of monooctyl-PEGs, as determined using Eq. 6

| Sample | Mass ($\mu$g) | | |
|---|---|---|---|
| | $R_8EO_4$ | $R_8EO_5$ | $R_8EO_{10}$ |
| PEG | 0.0 | 4.8 | 6.6 |
| Octanol | 0.0 | 26.0 | 0.0 |
| Octyl-PEG | 555.7 | 462.5 | 160.3 |
| Sum | 555.7 | 493.4 | 166.8 |
| True sample size | 549.0 | 489.0 | 176.0 |

## 6. Acknowledgement

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