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Liquid exclusion–adsorption chromatography, a new technique for isocratic separation of nonionic surfactants II. Quantitation in the analysis of fatty alcohol ethoxylates

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Abstract

A new technique of liquid chromatography, which allows baseline separation of fatty alcohol ethoxylates with up to 15–20 ethylene oxide units under isocratic conditions allows an accurate quantitative analysis of single hydrophobic chain surfactants. Using density and refractive index detection, the accurate weight fractions of the individual oligomers are obtained. Moreover, the contribution of preferential solvation can be determined. With refractive index detection alone, good accuracy can also be achieved.

Keywords: Liquid exclusion–adsorption chromatography; Detection, LC; Surfactants, non-ionic; Fatty alcohol ethoxylates; Ethoxylates

1. Introduction

As has been discussed in Part I of this series [1], different modes of liquid chromatography can be applied in the analysis of nonionic surfactants [2]:

(i) Size exclusion chromatography (SEC) separates according to molecular size (not actually molecular mass!). It is always performed in isocratic mode, typically in pure solvents.

(ii) Liquid chromatography at the critical point of adsorption (often also called LC under critical conditions; LCCC) separates according to structural units other than the repeating unit, i.e. end groups etc. LCCC is also run under isocratic conditions, but typically in mixed mobile phases.

(iii) Liquid adsorption chromatography (LAC) separates according to chemical composition and to molecular mass. In principle, LAC can also be performed using isocratic elution, but samples with higher molecular mass typically require gradients [3–15], which however make quantitation more or less problematic.

Depending on the nature of the samples (and the chromatographic technique), different detectors can be applied in chromatography of surfactants.

The most familiar detectors are the UV detector, which can, however, only be applied to samples absorbing light of a wavelength, for which the mobile phase is sufficiently transparent, and the Refractive Index (RI) detector. The density detector (according to the mechanical oscillator principle) is

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very useful in polymer analysis, especially in combination with other detectors. Both the density and RI detector can only be applied in isocratic elution.

Consequently, the analysis of samples without chromophores — such as fatty alcohol ethoxylates (FAEs) — by gradient elution faces a severe detection problem. In the last years, evaporative light scattering detector (ELSD) [16–18] has often be employed for such analytical tasks [4,19,20].

It is claimed to be a ''mass detector'', because is should detect any non-volatile material in any mobile phase composition.

Unfortunately, this is not true: the sensitivity of this instrument depends on various parameters [21] which cannot always be easily controlled, and its response to polymer homologous series is not as well understood as that of RI and density detection. It must be mentioned, that there are basically two different designs of the ELSD system: in the Sedex and DDL 21 instruments, the mobile phase is nebulized at room temperature in a special spray chamber, in which larger droplets are trapped, while in other types (PL, Varex, Alltech, DDL 31) the entire aerosol passes the heated drift tube, where volatile components are (more or less) evaporated. Obviously, the number and size of the droplets reaching the evaporator depend on the composition and the flow-rate of the mobile phase as well as on the flow-rate of the carrier gas [16,21], which all have a substantial impact on particle size after stripping off the solvent shell during the evaporation process. In a recent study [22], we have applied three different instruments to the same analytical task and compared their behavior. The most important results of this study were:

(i) The response of all three instruments was nonlinear with a different curve shape.

(ii) Lower oligomers ($n<4$) had very small response factors.

(iii) Lower fatty alcohols ($<C_{14}$) were not detected at all.

(iv) There was a strong influence of mobile phase composition on detector response!

While the nonlinear response can easily be corrected, it is rather difficult to compensate for the influence of mobile phase composition in gradient LAC, where RI detection cannot be applied: with density detection coupled to ELSD, the actual mobile phase composition for each peak can be determined. Compared to the huge density changes related to the gradient, the response to the sample and the contribution of preferential solvation are negligible. On the other hand, valuable information on the performance of the gradient pump is obtained.

These corrections are, however, laborious and require very careful calibrations.

Considering all these problems, one should prefer a separation under isocratic conditions. This can be achieved using a new separation mechanism, which has been described in detail in Part I of this series [1]: liquid exclusion–adsorption chromatography (LEAC). In a mobile phase composition, at which the ethylene oxide (EO) chain is eluted in the exclusion regime, while the alkyl groups are adsorbed, the individual oligomers are separated in the order of SEC, but far behind the void volume. A similar behaviour has also been observed in other systems [23–25], which indicates, that this technique can be applied to block copolymers in general.

A theoretical explanation is also presented in this paper [1]: the key parameters in such a separation of amphiphilic molecules AB are the pore diameter of the stationary phase, the radius of gyration R_A of the block in exclusion regime (A, i.e. the EO chain), and the adsorption interaction parameter c_B of the block in adsorption regime (B, in this case the alkyl group). For molecules much smaller than the pores, the distribution coefficient K_{AB} decreases with R_A in a similar way as in SEC. At high degrees of ethoxylation, however, K_{AB} tends to zero with $1/R_A$.

For samples with an average degree of ethoxylation up to 10, a baseline separation of the individual oligomers can be achieved.

In this study, the quantitative reliability of this technique should be evaluated.

2. Experimental

These investigations were performed using the density detection system DDS 70 (Chromtech, Graz, Austria), which has been developed in our group [26]. The DDS 70 was connected to a MS-DOS computer via the serial port.

Data acquisition and processing was performed using the software package CHROMA, which has

been developed for the DDS 70. The columns and density cells were placed in a thermostatted box, in which a temperature of 25.0°C was maintained for all measurements.

The mobile phase was delivered by a Jasco 880 PU pump (Japan Spectrosopic Company, Tokyo, Japan) at a flow-rate of 0.5 ml/min. Samples were injected manually using a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA, USA) equipped with an 50 μl loop.

A type Prodigy 5 μm ODS(3) column (250×4.6 mm, pore diameter 100 Å, ser 185970, Phemonenex, Torrance, CA, USA) was used for all measurements.

A Bischoff 8110 RI detector (Bischoff, Leonberg, Germany) was connected to the DDS 70. For comparative purpose, the RI detector was replaced in some measurements by a type Sedex 45 ELSD system (Sedere, Vitry sur Seine, France). Nitrogen was used as carrier gas, the pressure at the nebulizer was set to 1.0 bar and the drift tube temperature adjusted to 30°C.

The solvents used were HPLC grade. Acetone was purchased from Roth (Karlsruhe, Germany) and water from Riedel-de Haen (Seelze, Germany).

The following polydisperse FAE samples were used in these investigations (specifications given by the producer: Fluka, Buchs, Switzerland):

(i) Brij 52: polyethylene glycol hexadecyl ether, main component: diethylene glycol hexadecyl ether;

(ii) Brij 72: Polyethylene glycol octaadecyl ether, main component: diethylene glycol octadecyl ether;

(iii) Brij 76: Polyethylene glycol octaadecyl ether, main component: decaethylene glycol octadecyl ether.

Monodisperse samples (C_{12} to C_{18} monoalkylethers of di- to octaethylene glycol) as well as polyethylene glycol (PEG) and fatty alcohols were also purchased from Fluka.

3. Results and discussion

FAEs can be considered as functional oligomers with end groups, that are chemically different from the repeating units of the chain, or as 2-block copolymers (with polyoxyethylene as block A and the fatty alcohol as block B). Consequently, the response factors of the individual oligomers will depend on chemical composition, i.e. the degree of ethoxylation within each polymer homologous series.

This becomes clear from Fig. 1, which shows a separation of Brij 52, which was obtained by LEAC in acetone–water (75:25, w/w) with density, RI detection and ELSD.

As could be expected, the response factors of all three detectors for the individual oligomers vary considerably with molecular mass: While all peaks are positive in RI detection, the sign of the peaks change in density detection between the monoethoxylate and the fatty alcohol, which is eluted between the mono- and diethoxylate, as has been explained in part 1 of this series.

When RI detection is replaced by ELSD, a surprising result is obtained: In the used mobile phase, the fatty alcohol and the lowest ethoxylates are obviously overestimated. The opposite had been found in a chromatogram obtained by gradient LAC (from 100% acetone to acetone–water, 80:20) [1]. In this case, the fatty alcohol (which appeared in the isocratic section — i.e. in pure acetone) was underestimated!

Obviously, it is not feasible to determine the response factors for all individual oligomers, because monodisperse oligomers are commercially available only for the lowest degrees of ethoxylation (up to 6–8), and even these samples are not always sufficiently pure.

As the response of density and RI detection is clearly defined (which is not the case with the ELSD), this problem can be solved using different approaches [2,27–30].

3.1. Influence of molecular mass on detector response:

As the response factors of the RI and the density detector are closely related to specific properties (refractive index increment and apparent specific volume, respectively), their dependence on molecular mass is given by the relation [28]:

$$f_i = f_\infty + \frac{K}{M_i} \quad (1)$$

wherein f_i is the response factor of a molecule with the molecular mass M_i, f_∞ is the response factor of the chain without end groups (i.e. with high degree

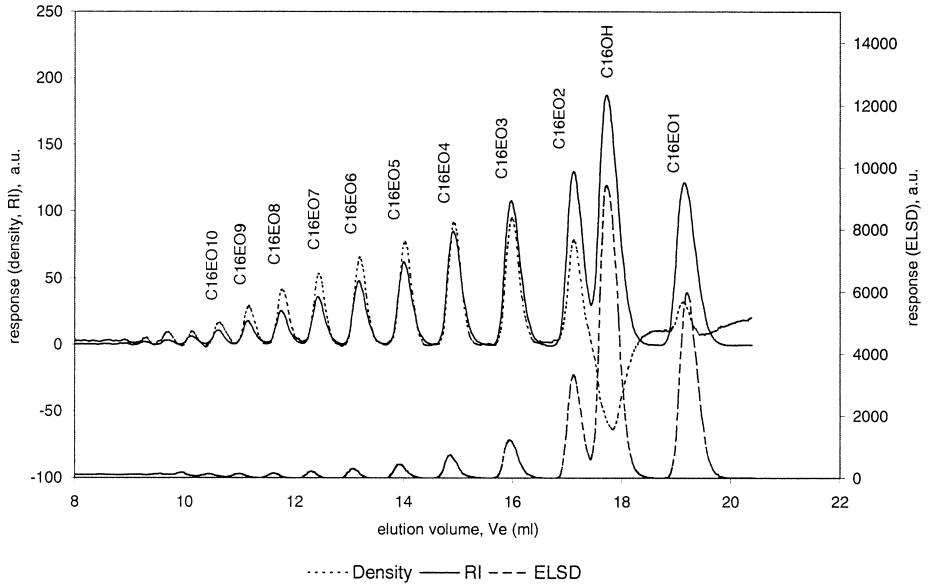

Fig. 1. Separation of Brij 52 by LEAC with different detectors. Sample size 444 μg, other chromatographic conditions as described in the Experimental section.

of polymerization), and K is a constant describing the influence of the end groups, which can be determined by linear regression (as is shown in Fig. 2) or simply by a two-point calibration using a sufficiently high-molecular-mass PEG and the fatty alcohol. As already mentioned, the response factor of the fatty alcohol is negative in density detection, while all others are positive.

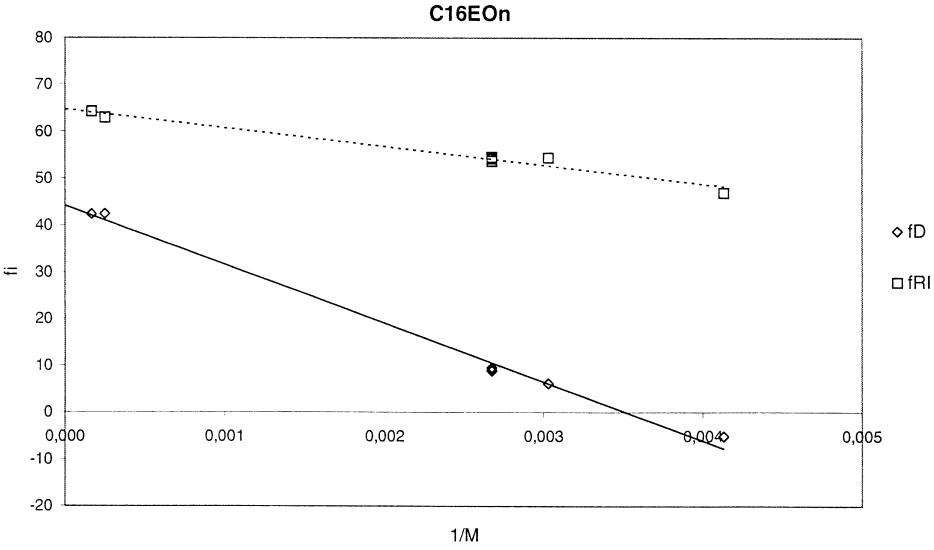

Fig. 2. Apparent response factors of PEG 6000, hexadecanol, and monodisperse oligoethylene glycol monohexadecyl ethers.

Once f_∞ and K are known, one may calculate the individual response factors for each oligomer with known M_i.

3.2. Influence of chemical composition on detector response [28,29,31]

Considering FAE as copolymers consisting of the components A (PEG) and B (fatty alcohol), a peak containing the mass m_i of an oligomer with the weight fractions w_A and w_B of the components A and B will have the area $x_{i,j}$ in the detector j (at the flow-rate F):

$$x_{ij} = m_i(w_A f_{j,A} + w_B f_{j,B})/F$$
$$= m_i \cdot [w_A(f_{j,A} - f_{j,B}) + f_{j,B}]/F \quad (2)$$

wherein $f_{j,A}$ and $f_{j,B}$ are the response factors of density and RI detector for the fatty alcohol and a sufficiently high-molecular-mass PEG.

If two detectors are combined, the sensitivity of which for fatty alcohol and PEG is sufficiently different (such as density and RI detector), one may calculate the chemical composition for each peak from the ratio of the peak areas:

$$\frac{1}{w_A} = 1 - \frac{(\frac{x_{i,1}}{x_{i,2}} \cdot f_{2,A} - f_{1,A})}{(\frac{x_{i,1}}{x_{i,2}} \cdot f_{2,B} - f_{1,B})} \quad (3)$$

and therefrom the correct amount of the sample:

$$m_i = \frac{x_{i,1}}{w_A \cdot (f_{1,A} - f_{1,B}) + f_{1,B}} \quad (4)$$

As can be seen from Fig. 3, the chemical compositions obtained for the individual oligomers from the chromatogram of Brij 52 ($C_{16}EO_n$), which has been shown in Fig. 1, agrees quite well with the calculated ones.

3.3. Influence of preferential solvation on detector response

These considerations are, however, strictly valid only in pure mobile phases. In mixed mobile phases, one has to take into account, that polymers and oligomers show preferential solvation. If a polymer is dissolved in a mixed solvent, the composition of the solvent within the polymer coil may be different from outside. If such a solution is injected onto a

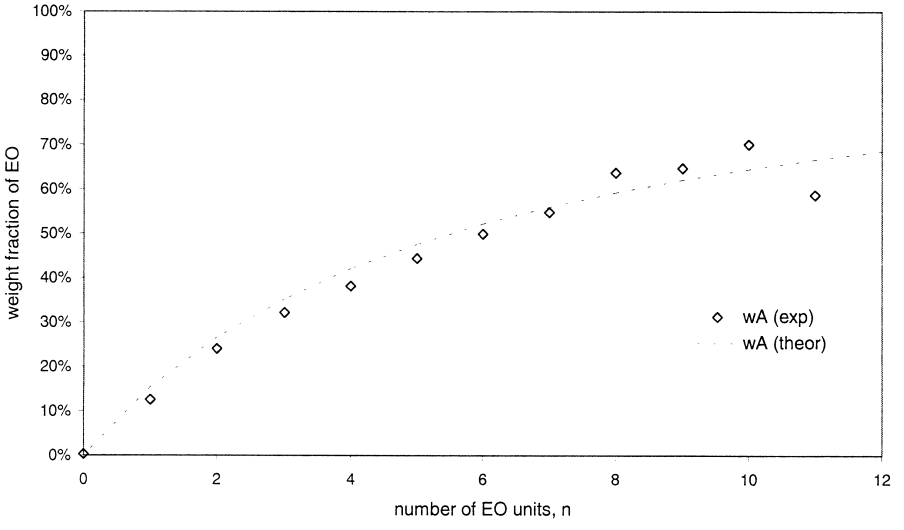

Fig. 3. Chemical composition of the individual oligomers in Brij 52, as obtained from the chromatogram shown in Fig. 1 with coupled density and RI detection using apparent response factors.

chromatographic column, the zone of ''dialyzed'' solvent is separated from the polymer peak, and a vacancy peak appears at the void volume (where the solvent would appear). As we have shown in a previous paper, this effect may depend on the molecular mass of the sample [32], and consequently, also on chemical composition. Obviously, the extent of preferential solvation will depend on the hydrophilic–lipophilic balance (HLB) [33] of amphiphilic molecules like FAEs.

Hence one has to distinguish between the apparent response factors, which are observed in chromatographic separations in presence of preferential solvation, and the true ones, which are observed, when the sample is injected directly without chromatographic separation. Obviously, the true factors are also obtained from the sum of polymer peak and the solvent peak.

In the case of PEG 6000, which is eluted in LEAC in the exclusion regime, the polymer takes up water from the mobile phase (acetone–water, 75:25, w/w), and a negative (water) peak appears. Obviously, the water missing there is eluted with the PEG, thus contributing to its peak area! (Fig. 4).

With fatty alcohols this effect is not observed in RI detection: in a chromatogram of tetradecanol (Fig. 5) the water peak is negligible. With density detection, which is quite insensitive for fatty alcohols, but highly sensitive for water, the water peak appears. which is, however, considerably smaller than in the chromatogram of PEG.

The contribution of preferential solvation for fatty alcohol ethoxylates will lie between these extremes and should increase with the degree of ethoxylation.

In a previous paper we have shown [34], that the amount of sample and water can be determined by a combination of density and RI detection, if the response factor of the individual polymer fraction in a peak is known. In LCCC, this is typically not the case, because a peak contains unknown amounts of an entire polymer homologous series (of unknown composition) and solvent. In this case, each fraction has to be analyzed by dual detector SEC, from which the chemical composition (and thus the average response factor) of the polymer can be obtained [34]

In LEAC this is much easier, as the individual oligomers can be separated and identified. Hence one can determine the amount of polymer and water in a peak, if the individual (true) response factors are known.

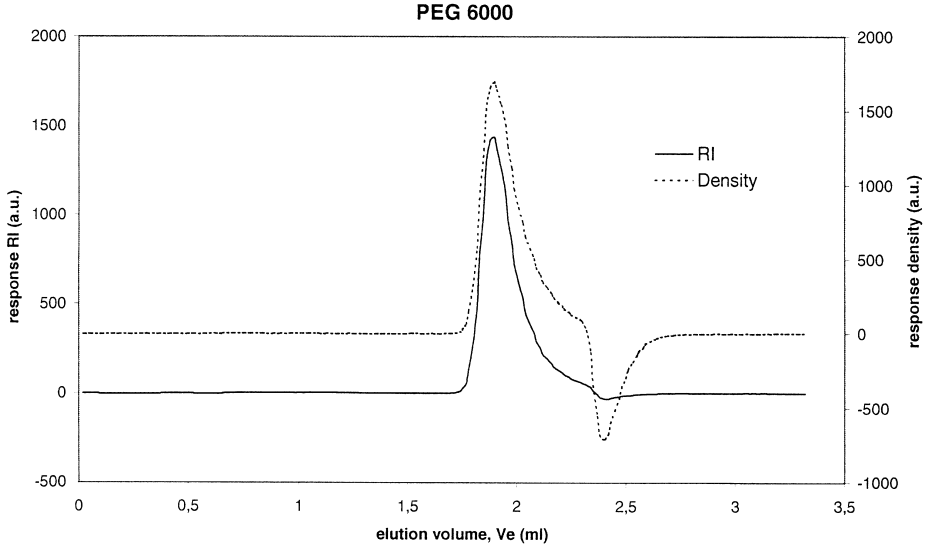

Fig. 4. LEAC of PEG 6000 with density and RI detection. Sample size 266 μg, other chromatographic conditions as described in the Experimental section.

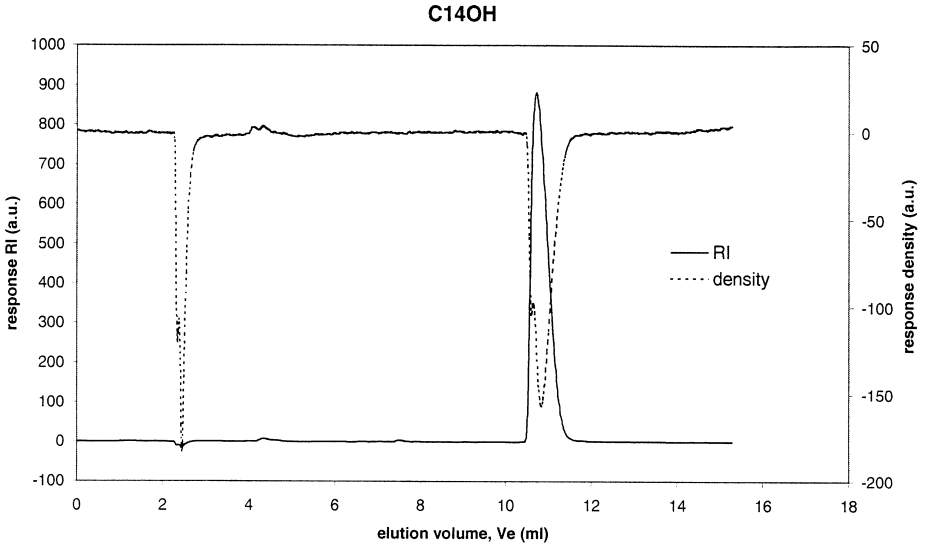

Fig. 5. LEAC of tetradecanol with density and RI detection. Sample size 545.5 μg, other chromatographic conditions as described in the Experimental section.

The mass m_P of the polymer or oligomer with the degree of polymerization i (the i-mer) in a peak is given by [34,35]:

$$m_P = F \cdot \frac{x_D f_{S,R} - x_R f_{S,D}}{f_{i,D} f_{S,R} - f_{i,R} f_{S,D}} \quad (5)$$

and the amount m_S of solvent (in this case water) from preferential solvation by:

$$m_S = F \cdot \frac{x_R f_{i,D} - x_D f_{i,R}}{f_{i,D} f_{S,R} - f_{i,R} f_{S,D}} \quad (6)$$

When fatty alcohols with different carbon number are analyzed with dual detection, the correct amount of the fatty alcohol is obtained (using Eq. (5)), and the amount of water in each peak can be determined (using Eq. (6)).

The results are given in Fig. 6: as expected, preferential solvation increases with decreasing carbon number (i.e. increasing $1/M$).

In Fig. 7, the apparent and the true response factors of PEG, the fatty alcohol ($C_{14}OH$), and monodisperse FAE ($C_{14}EO_n$) are plotted as a function of $1/M$.

As can be seen, straight lines are obtained in both cases, which almost coincide for the RI detector, while they have a considerably different slope for the density detector.

3.4. Quantitation in the analysis of FAEs by LEACs

Basically there are three different approaches to obtain quantitatively reliable results in LEAC of nonionic surfactants:

(A) Assuming preferential solvation to be the same at different concentrations and in presence of other oligomers, one may calculate the apparent response factors from molecular mass (using Eq. (1)) for the individual oligomers in single mode detection, which will be preferably RI detection.

(B) Using dual detection with the apparent response factors, one may calculate the chemical composition for each peak without any assumption about molecular mass [Eq. (3)] and therefrom the amount ([Eq. (4)].

(C) With dual detection and the true response factors, one may determine independently the amount of each oligomer and water in each peak [Eqs. (5) and (6)].

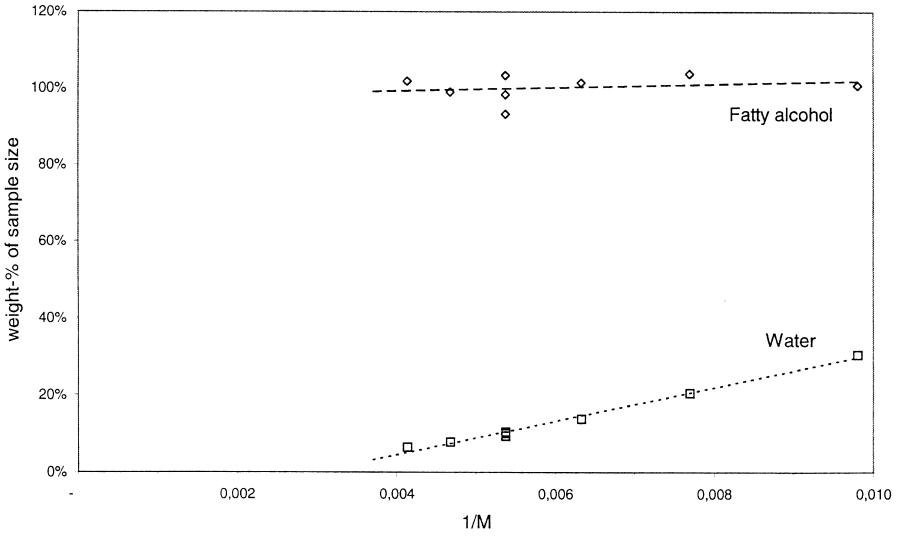

Fig. 6. LEAC of fatty alcohols: found amount of fatty alcohol and water from preferential solvation (in % of sample size).

In Fig. 8, a chromatogram of a monodisperse sample ($C_{14}EO_6$) is shown. As can be seen, the RI detector is very insensitive towards water in the mobile phases used in this study, while large (negative) water peaks can be observed with the density detector.

As can be seen from Fig. 9, the different approaches agree very well: only with the density detector alone, the results scatter considerably for the lowest oligomers (according to their low response factors).

With dual detection (using the true response factors), the extent of preferential solvation can be determined (or at least estimated) for the individual oligomers, as can be seen from Fig. 10.

There are, however, some limitations: Fig. 11

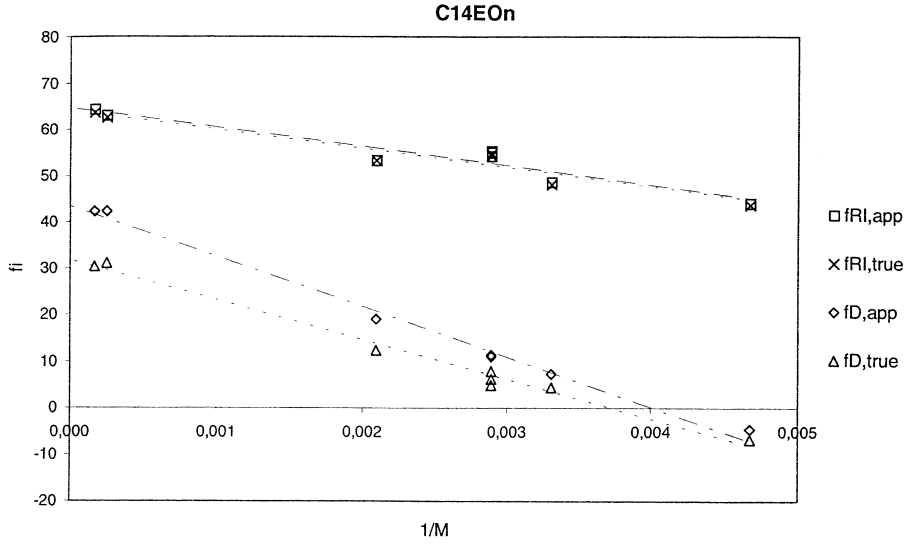

Fig. 7. True and apparent response factors of PEG 6000, tetradecanol, and monodisperse oligoethylene glycol monotetradecyl ethers in acetone–water (75:25, w/w) at a flow-rate of 0.5 ml/min.

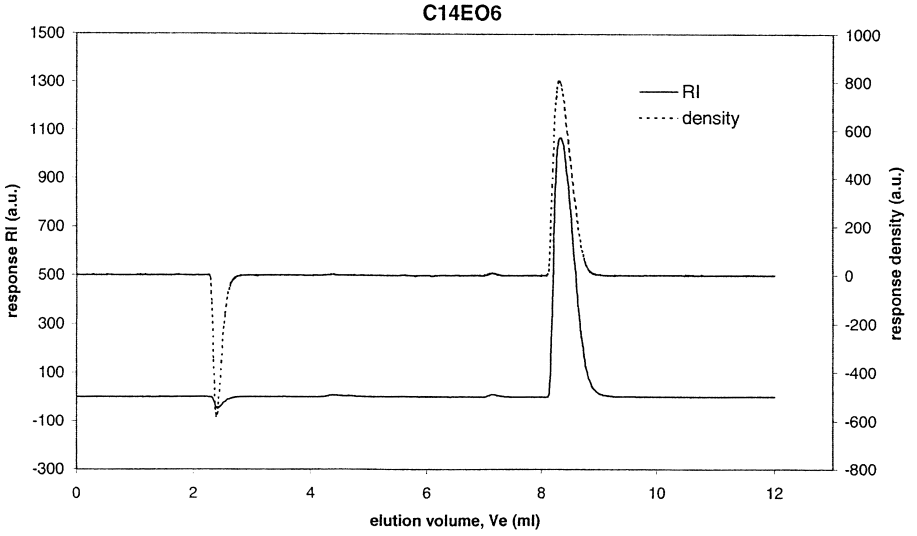

Fig. 8. LEAC of hexaethylene glycol monotetradecyl ether ($C_{14}EO_6$) with density and RI detection. Sample size 459 μg, other chromatographic conditions as described in the Experimental section.

shows a chromatogram of Brij 76, in which about 25 peaks are quite well separated. At lower elution volumes, however, there is a fraction, which does not belong to the same polymer homologous series (as will be shown in another communication, this sample contains several percent of C_{16} ethoxylates). Obviously, these two series of peaks overlap. As the amount of the C_{16} fraction is rather small, it does not affect the result of the C_{18} series very much (Fig. 12).

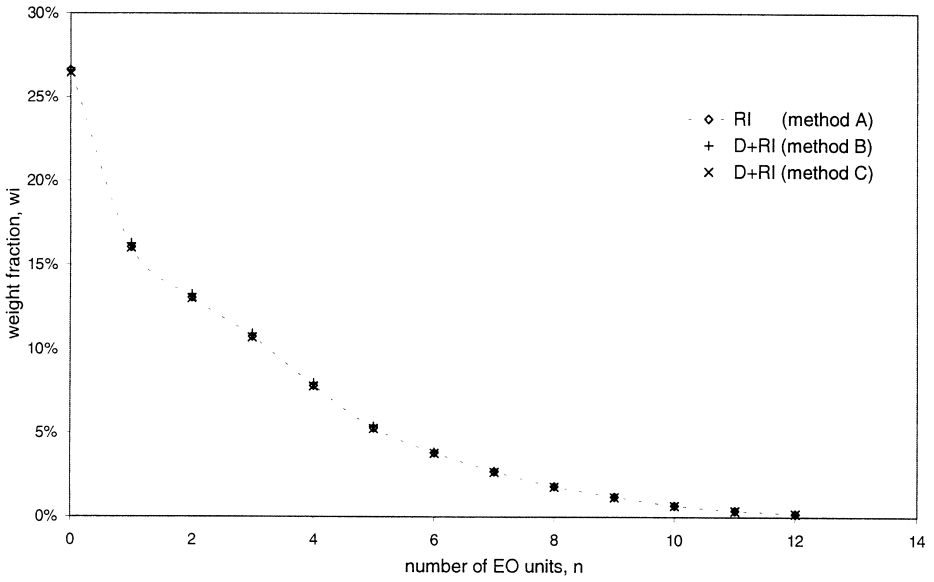

Fig. 9. Oligomer distribution of Brij 52, as determined by LEAC (Figure1) with density and RI detection using different approaches (A–C). Chromatographic conditions as described in the Experimental section.

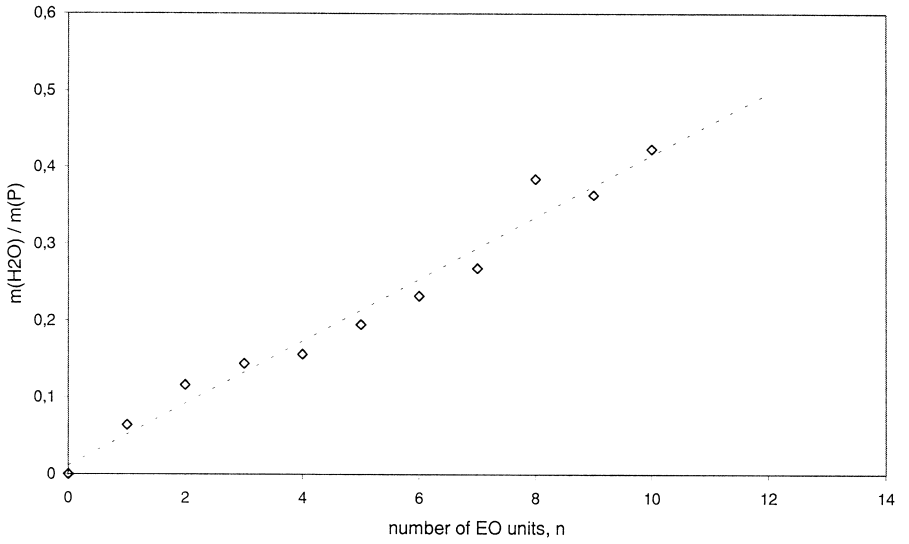

Fig. 10. Preferential solvation in LEAC of Brij 52, as obtained from the chromatogram shown in Fig. 1 with dual detection using Eqs. (5) and (6).

Consequently, samples containing larger amounts of other polymer homologous series must be separated by LCCC in the first dimension before analyzing them by LEAC.

As will be shown in another paper, two-dimensional LC with LCCC as the first and LEAC as the second dimension will be the method of choice in the analysis of FAEs with low to medium degree of ethoxylation.

4. Conclusions

Fatty alcohol ethoxylates consisting of a single

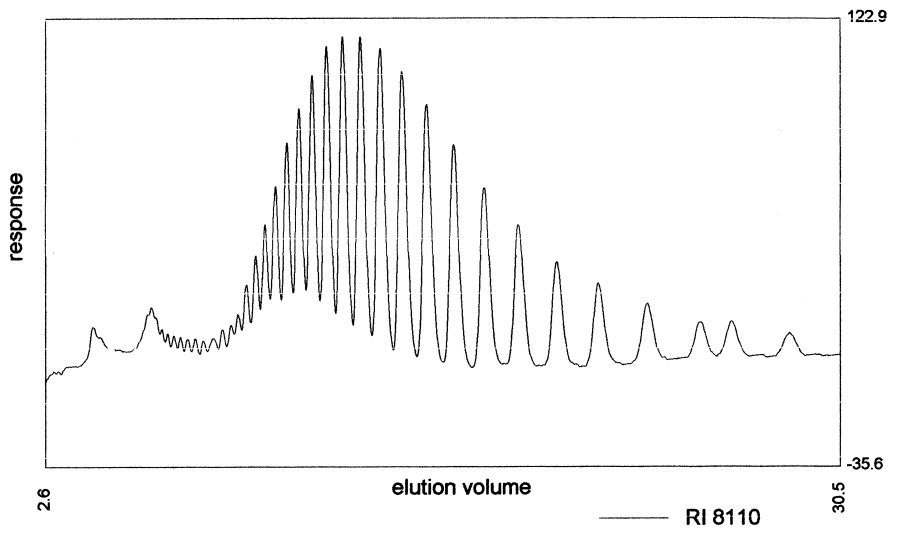

Fig. 11. Separation of Brij 76 by LEAC with different detectors. Sample size 618 μg, other chromatographic conditions as described in the Experimental section.

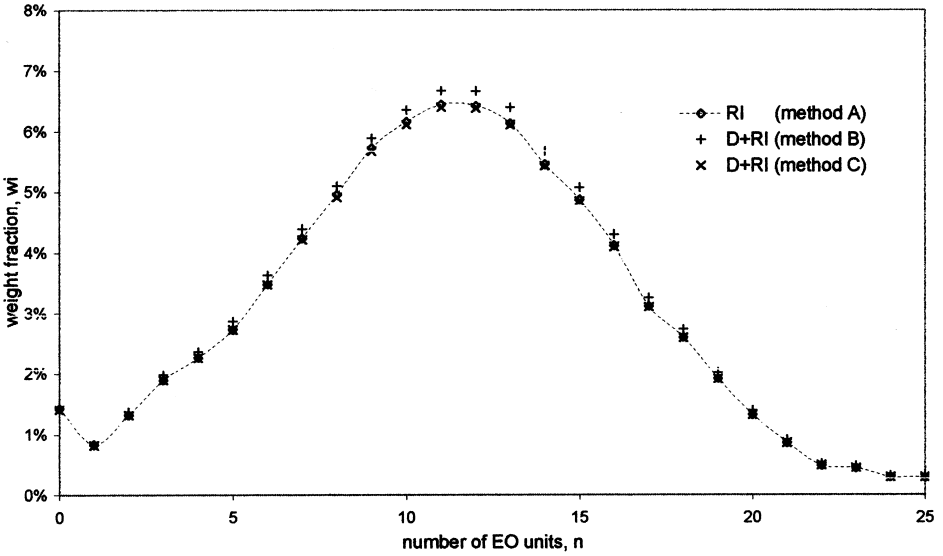

Fig. 12. Oligomer distribution of Brij 76, as determined by LEAC (Fig. 11) using different approaches (A–C).

polymer homologous series can be analyzed by LEAC under isocratic conditions with density and RI detection. This technique allows a baseline separation of the individual oligomers up to a number of 15–20 oxyethylene units, and an accurate quantitative determination of the amount of each oligomer and of the solvent it takes up by preferential solvation. Good accuracy can even be achieved by the simplest approach using RI detection alone with the apparent response factors.

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