Molecular Characterization of Functional Polymers and Oligomers by Liquid Chromatography

Bernd Trathnigg[a]*, *Christina Rappel*[a], *Renate Hoedl*[a], *Alexei Gorbunov*[b]

[a] Institute of Chemistry, Karl-Franzens-University, A-8010 Graz, Austria
[b] Institute for Highly Pure Biopreparations, Pudozhskaya 7, 197110 St.Petersburg, Russia,

SUMMARY: Different chromatographic regimes can be applied to the separation of functional polymers and oligomers: Size Exclusion Chromatography (SEC), Liquid Adsorption Chromatography (LAC), Liquid Chromatography under Critical Conditions (LCCC) and Liquid Exclusion-Adsorption Chromatography (LEAC). All oligomers of non-ionic surfactants can be separated to the baseline by combination of these techniques. In some cases, gradient elution is required to separate all possible homologous series.

Introduction

Polymers and oligomers containing polyoxyethylene block and functional end groups are used for many purposes.

Fatty alcohol ethoxylates (FAE) are commonly used as nonionic surfactants. Fatty acid methyl ester ethoxylates (FAMEE) [1,2] are new nonionic surfactants, which can be produced by the direction of ethylene oxide (EO) to fatty methyl esters (FAME) in the presence of a composite metal oxide catalyst. FAMEE have been compared to common FAE with respect to surface-chemical and physico-chemical properties [3,4]: FAMEE and FAE have similar surface tension-lowering characteristics.

Fatty acid polyglycol esters are amphiphilic compounds. They are used as emulsifiers in many applications, such as cosmetic and care products and in textile fabrication, as anti-static lubricants for textile auxiliaries and as viscosity regulators in surfactant formulations. These materials can be obtained either by ethoxylation of fatty acids [5-8] or by esterification of polyethylene glycols [5,8]. In both cases they typically contain different homologous series of monoesters (depending on the purity of the fatty acid used as starting material) and the corresponding series of diesters (eventually also polyethylene glycol and fatty acids).

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As the properties of all these products depend strongly on their molecular weight distributions and chemical compositions, as well as on their architecture, there is a strong need for reliable analytical methods by which such products can be fully characterized. Amphiphilic polymers can generally be characterized using different chromatographic techniques, which separate according to different criteria.

1. The overall molar mass distribution (MMD) can be determined by Size Exclusion Chromatography (SEC).
2. The individual homologous series can be separated according to the non-polar component by Liquid Chromatography at the Critical Adsorption Point (CAP) for the (polar) polyoxyethylene block on a reversed phase column (Liquid Chromatography under Critical Conditions : LCCC) [9-14].
3. A separation according to the polar component can be achieved by Liquid Adsorption Chromatography (LAC) [15-17] on a normal phase column or by Liquid Exclusion-Adsorption Chromatography (LEAC) [18,19] on a reversed phase column. In the range of lower degrees of ethoxylation LEAC is superior to LAC, as it can be run in isocratic mode, while LAC typically requires gradient elution, which has accompanying detection problems [20-24].

Obviously, a full characterization of complex samples requires a combination of at least two different separation modes, such as LCCC-SEC, LCCC-LAC, or LCCC-LEAC.

Theory

The elution volume of a species in liquid chromatography is given by

$$V_e = V_i + KV_p \tag{1}$$

where V_i denotes the interstitial volume (i.e. the volume of the solvent outside the particles of the packing), V_p is the entire pore volume, and, consequently, $V_0 = V_i + V_p$ is the void volume of the column. K is the distribution coefficient relevant to the partition function of a polymer chain within an in-pore space.

In size exclusion chromatography (SEC), which is governed by entropy, $0 < K < 1$.

In adsorption chromatography (LAC), the (enthalpic) interactions with the stationary

phase are strong, and K increases exponentially with the number of repeating units, hence $K >> 1$.

In LCCC, entropic and enthalpic terms compensate each other the so-called critical adsorption point (CAP) is defined by a mobile phase composition and temperature, at which non-functional molecules are eluted (regardless their molar mass) at the void volume ($V_0 = V_i + V_p$). Hence their distribution coefficient equals unity: $K^{(0)} = 1$.

These regimes may also be classified in terms of the adsorption interaction parameter c:

In SEC, c is negative, in LCCC, c = 0, and in LAC, c is positive. The following considerations concern the elution behaviour of functional molecules at the CAP.

For monofunctional molecules (with one end group *a*) an additionaly term q_a appears, which accounts for the difference between the end group and the repeating units.

The distribution coefficient $K^{(a)}$ is then given by

$$K^{(a)} \approx 1 + q_a \tag{2}$$

Consequently, the distribution coefficient $K^{(aa)}$ of symmetrical difunctionals with groups *a* at both ends is given by

$$K^{(aa)} \approx 1 + 2q_a + q_a^2 \cdot \frac{d}{\sqrt{\pi} \cdot R} \tag{3}$$

which means that a separation according to the radius of gyration (R) can be achieved.

In the more general case of asymmetrical difunctionals having different end-groups, *a* and *b*, the distribution coefficient equals:

$$K^{(ab)} \approx 1 + q_a + q_b + q_a \cdot q_b \cdot \frac{d}{\sqrt{\pi} \cdot R} \tag{4}$$

As follows from eqs. (2-4), straight lines should be obtained in a plot of the distribution coefficient versus $1/R$. From their intercept, the interaction parameters of the end groups can be obtained. Obviously, the slope should equal zero in non- and monofunctionals, and it should be positive for difunctionals, if the parameter q of both end groups has the same sign.

If the composition of the mobile phase deviates from the critical one, one may obtain a separation of amphiphilic molecules, provided that the interaction parameter of the repeating unit, c_A, is negative, while that of the end group (or a second block in a diblock copolymer), c_B, is positive. For small lengths of the EO chain B (small R_A) one obtains

$$K_{AB} \approx K_B(1-\frac{\sqrt{\pi}}{2}c_B R_A) = K_B(1-\tilde{C}\sqrt{M_A}). \quad (5)$$

Equation (5) shows the same elution order as in SEC, but the values of K are more than unity, which is characteristic of the LAC mode. That is why we apply the term 'liquid exclusion-adsorption chromatography' (LEAC) to such a type of chromatographic separation.

Combinations of the mechanisms decribed above have been successfully applied by us in the separation of non-ionic surfactants.

Experimental

Investigations were performed using the density detection system DDS70 (CHROMTECH, Graz, Austria), which has been developed in our group. All measurements were performed at a flow rate of 0.5 ml/min and a constant temperature of 25.0° C. Two other detectors were connected to the DDS 70: A Bischoff 8110 RI detector (Bischoff, Leonberg, Germany) and a Sedex 45 ELSD apparatus (Sedere, Vitry sur Saine, France). Nitrogen was used as the carrier gas, the pressure at the nebulizer was set to 1.0 bar and the temperature of the evaporator to 30°C .

The following columns were used in these investigations:

Zorbax 300 C18 (150x4.6 mm, 3.5 µm, 300 Å, from Rockland Technologies)
Prodigy 5µ ODS3 (250x4.6 mm, 5 µm, pore diameter 100 Å, from Phenomenex)
Symmetry C18 (150x3.0 mm, 5 µm, from Waters).
The solvents (acetone, methanol and water, all HPLC grade) were purchased from Roth, Karlsruhe, Germany). The mobile phase compositions are given in weight %.

The following polydisperse samples were used in our investigations (specifications given by the producer). Polyethylene glycol monolaurate (average molecular masses

400) was obtained from Sigma-Aldrich. Fatty acid methyl ester ethoxylates (FAMEEs) and lauric acids ester ethoxylates were provided by W. Hreczuch, ICSO, Kedzierzyn-Kozle, Poland. Monodisperse mono- and diesters of oligoethylene glycols were prepared by esterification of the fatty acids in toluene, using p-toluenesulfonic acid as a catalyst [8].

Results and Discussion

As has already been shown in previous papers, LEAC can provide a baseline separation of individual oligomers in nonionic surfactants, with up to 20 EO units, under isocratic conditions, which allows an accurate quantitation (from RI detection).

An example is given in the following figure, which shows the separation of a FAMEE with an overall degree of ethoxylation of 4.

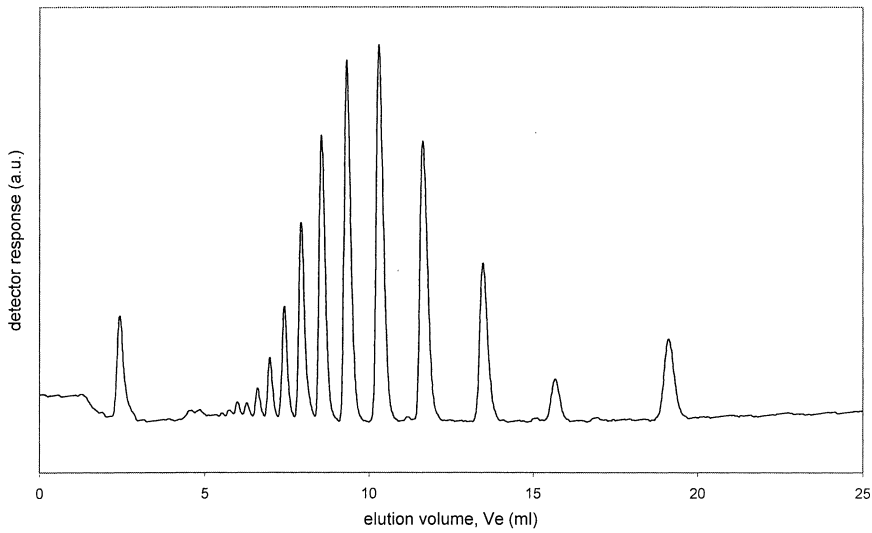

Figure 1. LEAC of a fatty ester ethoxylate (based on methyl laurate) with an average degree of ethoxylation of N=4, as obtained on Prodigy ODS3 in 71.5 % acetone. Detection: RI

If the composition of the mobile phase corresponds to the CAP, the monoesters in fatty acid ethoxylates appear as narrow peaks, while the diesters are separated in the order of decreasing radius of gyration. The ethylene glycol diester appears as the last peak in the

chromatogram (Fig. 2).

If the separation is started under LEAC conditions for PEG, the diesters are strongly adsorbed or even precipitated on the top of the column, while the monoesters are separated as above. Applying a step gradient from the LEAC phase to the mobile phase composition used in Figure 2, the diesters are also separated to the baseline (Fig. 3).

Samples containing more than one series of mono- and difunctionals cannot be analyzed the same way. In this case, 2D-LC is required: the first step must be a separation of the monofunctionals by LCCC and the fractions thus obtained must be transferred to LEAC (preferrably using the FAD technique, as has been described previously).

Figure 4 shows the result of such a separation of the monofunctionals in a technical PEG-ester.

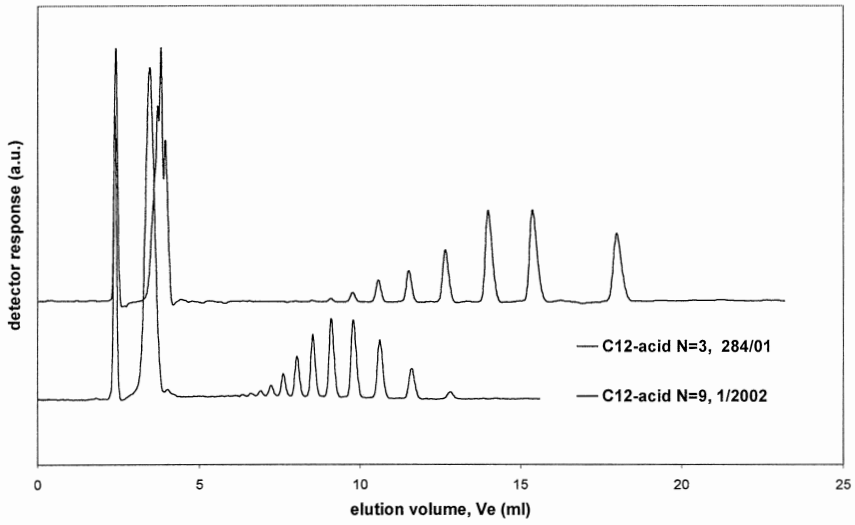

Figure 2. Separation of two lauric acid ethoxylates (with average degrees of ethoxylation N=3 and N=9) on Prodigy ODS3 close to the CAP. Chromatographic conditions: 85.3 % acetone, 0.5 ml/min, injected volume 20 µl. Detection: ELSD, evaporator temperature: 30°C.

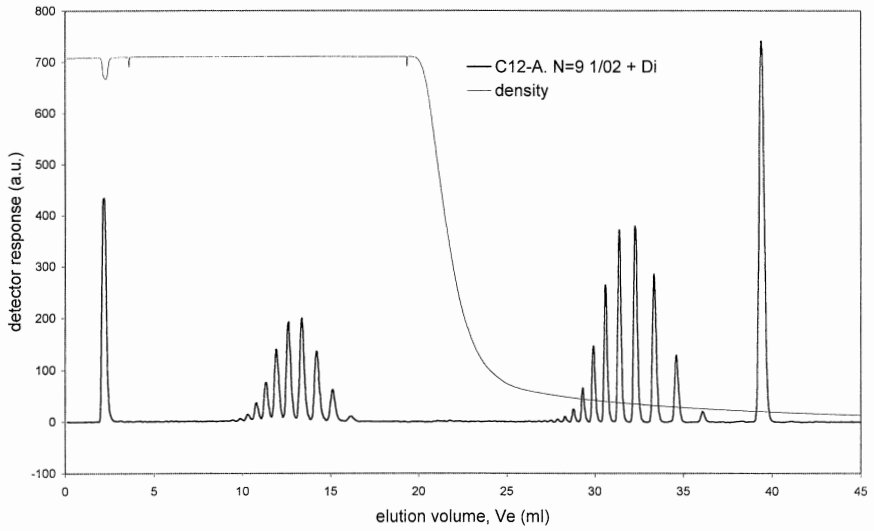

Figure 3. Separation of a lauric acid ethoxylate on Prodigy ODS3 with a step gradient. Chromatographic conditions: A: 57.3 % acetone, B: 85.3 % acetone, 0.5 ml/min, injected volume 20 µl. Detection: ELSD, evaporator temperature: 30°C

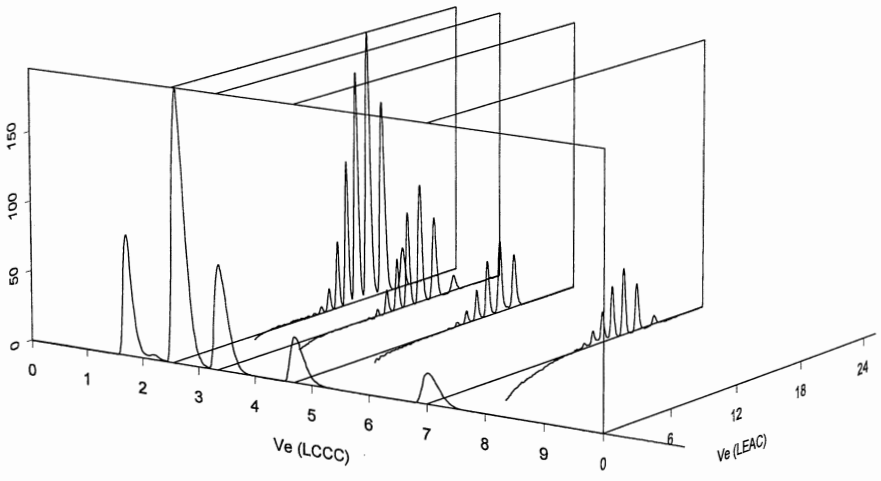

Figure 4. 2D-separation of PEG monolaurate 400 using LCCC as the first and LEAC as the second dimension and a full-adsorption-desorption column (FAD) for focusing of fractions.

In the first dimension, the difunctionals appear at quite high elution volumes, which are not shown in this figure. As the sample shown here is based on a technical fatty acid containing C12, C14, C16, and C18 acids, one can expect that there will be symmetrical and non-symmetrical diesters present. Since the retention of these may be different, we synthesized a symmetrical diester of diethylene glycol with two C14 end groups, and an unsymmetrical one with C12 and C16 end groups. Of course, the second one contains also some C12-C12 and C16-C16 diesters. Comparison of the chromatograms obtained with these diesters shows that the peak of the C12-C16 diester practically coincides with that of the C14-C14 diester. If these diesters are both present in the sample they will merge into a single peak in the chromatogram.

To prove whether the same is true for all pairs of corresponding symmetrical and non-symmetrical diesters that are expected to present in a technical PEG monolaurate 400 sample, we applied the theory to simulate a chromatogram for the polydisperse mixture of such diesters as can be seen in Figure 6.

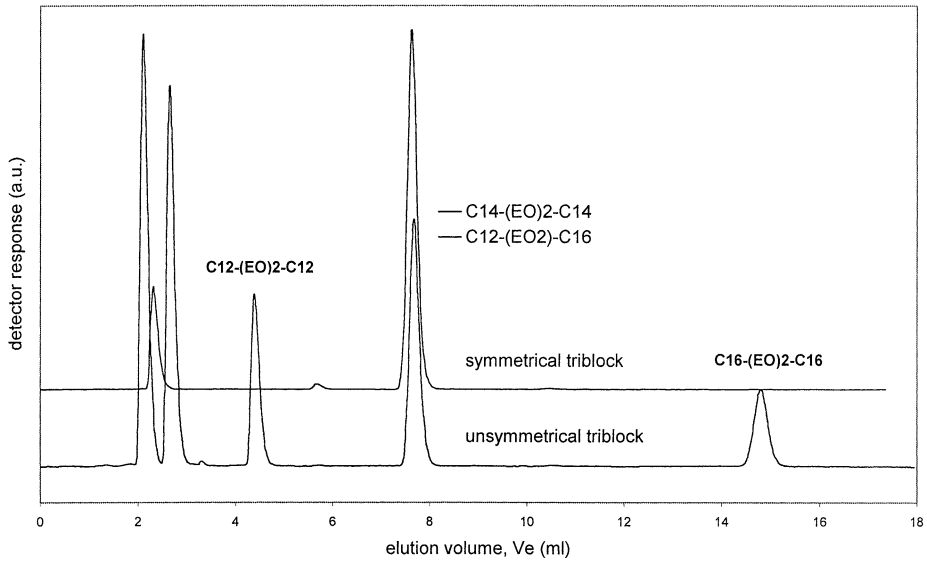

Figure 5. LCCC of symmetrical and unsymmetrical diesters of diethylene glycol, as obtained on the Zorbax column in 95 % methanol

In this simulation we used, for all individual molecules, the same values of the two

parameters accounting for the interaction of EO- and CH_2- units with the stationary phase. These values of the two interaction parameters for the Zorbax column and methanol-water (95% methanol) were estimated from independent chromatographic data obtained with PEG and PEG-monoesters. Simulation confirmed what was found experimentally (Fig. 5), namely that under the given experimental conditions symmetrical and corresponding non-symmetrical PEG-diesters appear in the chromatogram at about the same positions. Thus, in LCCC symmetrical and slightly unsymmetrical oligomeric diesters are separated, regardless of their architecture, according to the total number of CH_2 groups in their terminal fragments and to the number of repeating EO-units. Figure 7 shows the chromatogram obtained for PEG monolaurate 400 under isocratic conditions. Apart from the narrow initial peaks (PEG and PEG- monoesters), a five-peak series, representing different PEG-400 diesters, was completely separated in the chromatogram. It is clear from the above discussion and from the comparison of the real chromatogram with the simulated one, that the three last series of peaks in Figure 6 may either correspond to symmetrical or to non-symmetrical PEG-400 diesters or represent a mixture of such molecules.

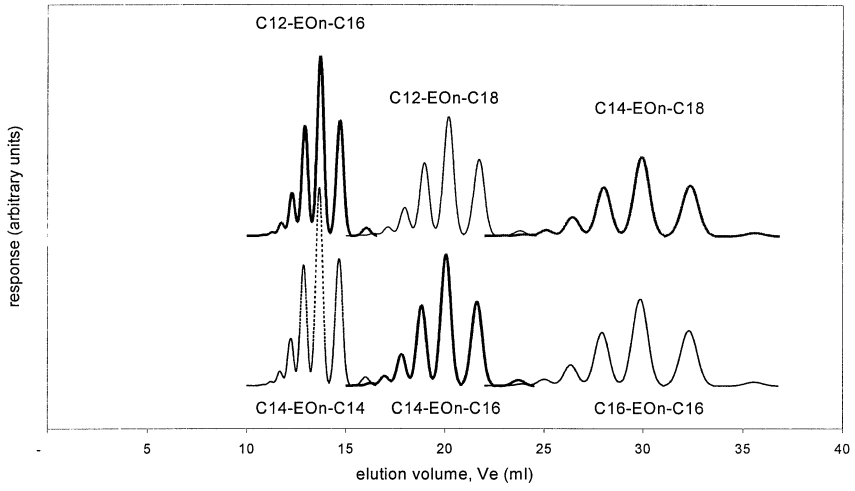

Figure 6. Simulated chromatogram for the mixture of symmetrical and unsymmetrical PEG-400 diesters. Simulation parameters: pore diameter 20.9 nm, void volume 1.72 ml, pore volume 0.73 ml, efficiency 6000, logarithmically normal molar mass distribution of PEG-block with Mw=184, and Mw/Mn=1.07, ineraction parameter for EO unit 0.16 nm^{-1}, interaction parameter for CH_2-unit 2.94 nm^{-1}

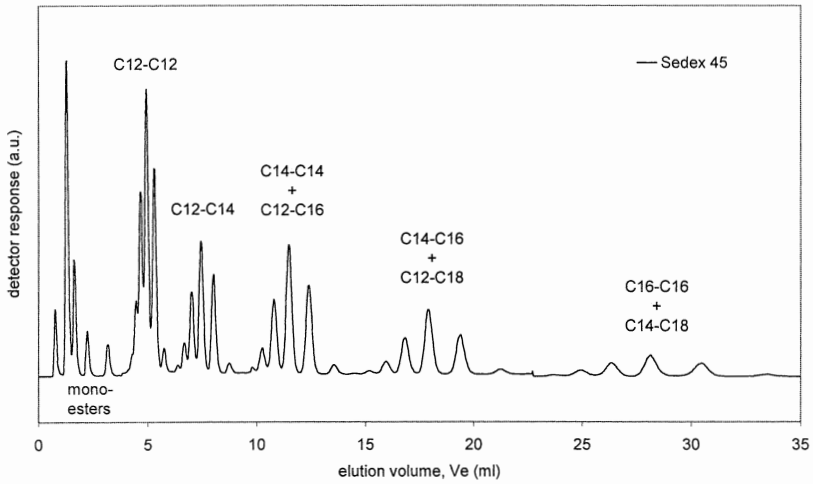

Figure 7. LCCC of the lower diesters in PEG monolaurate 400, as obtained on the Symmetry column in 95 % methanol. The sensitivity of ELSD was increased after the monoesters eluted.

If C12-C18 acids are present in the starting material, a total of 7 series of peaks should be expected. These were indeed detected when gradient elution was applied (Figure 8).

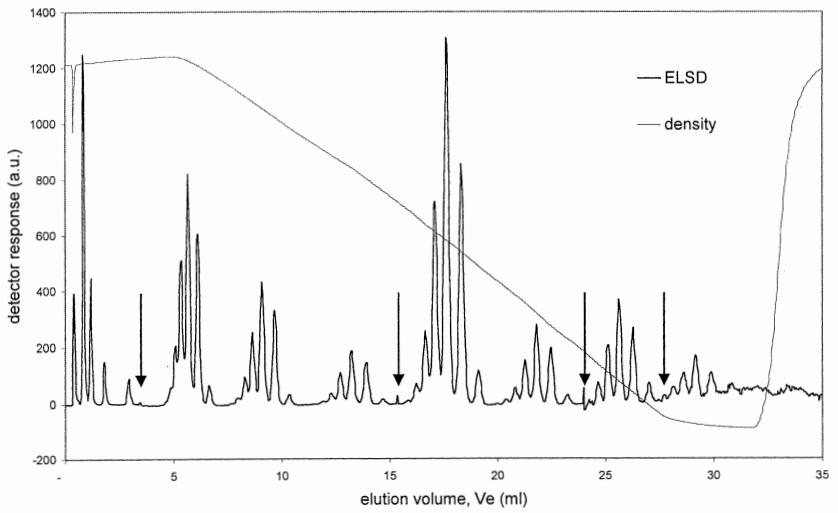

Figure 8. Gradient elution of the diesters in PEG monolaurate 400, as obtained on the Symmetry column. Gradient: from 90 % to 100 % methanol in 23 min. Sensitivity of ELSD was increased at the position indicated by the arrows

It must be pointed out, that in this case – unlike in all other polymer separations, where the higher molecular weights are more strongly retained than the lower ones - the gradient was used to speed up the elution of the lowest oligomers !

Conclusions

Ethoxylates of fatty acids and fatty esters can be successfully analyzed by liquid chromatography in different regimes. Monofunctionals (which can also be considered as two-blocks) are separated to the baseline by LEAC, which is run under isocratic conditions, allowing an accurate quantitation using the RI detector. Difunctionals (which can also be considered as three-blocks) are separated even at the CAP by a different mechanism. Samples containing mono- and diesters of only one fatty acid can be analyzed in a single run using a step gradient from LEAC to LCCC conditions.

Samples containing several polymer homologous series (with different fatty acids at the chain ends) generally require a two-dimensional separation. The difunctionals can, however, be fully analysed by using LCCC only. It is shown both experimentally and theoretically that oligomeric symmetrical and slightly unsymmetrical diesters (regardless their architecture) can be separated by LCCC according to the number of EO units and to the total number of CH_2 groups in their terminal fragments. If more than three fatty acids are present, gradient elution is required to analyze all of the diesters.

Acknowledgements

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