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Liquid exclusion–adsorption chromatography: a new technique for isocratic separation of non-ionic surfactants V. Two-dimensional separation of fatty acid polyglycol ethers

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Abstract

Fatty acid polyglycol esters can be fully characterized using two-dimensional liquid chromatography with liquid chromatography under critical conditions (LCCC) as the first and liquid exclusion–adsorption chromatography (LEAC) as the second dimension. LEAC is run under isocratic conditions, which allows the use of the refractive index detector, and thus accurate quantitation. Fractions from LCCC are transferred to LEAC using the full adsorption–desorption technique, by which they are focussed and reconcentrated before injection into the second dimension. This is achieved by increasing the water content of the mobile phase behind the LCCC column. Monoester oligomers of up to 20 oxyethylene units can be resolved to the baseline. Diester oligomers are partially separated in the first dimension (LCCC). © 2002 Published by Elsevier Science B.V.

Keywords: Liquid exclusion–adsorption chromatography; Liquid chromatography under critical conditions; Two-dimensional liquid chromatography; Surfactants; Fatty acid polygycol ethers; Poly(ethylene glycol)

1. Introduction

Fatty acid polyglycol esters are amphiphilic compounds, which are used as emulsifiers in many applications, such as cosmetic and care products and in textile fabrication. They serve 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 [1–4] or by esterification of polyethylene glycols [1,4]. In both cases they will 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 polyethylene glycol (PEG) and fatty acids).

As the properties depend strongly on the chemical composition, reliable analytical methods are needed to allow full characterization of such products. Basically, amphiphilic polymers can be characterized using different chromatographic techniques, which separate according to different criteria.

(1) The overall molecular mass can be determined by size exclusion chromatography (SEC).

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(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) [5–10].
(3) Separation according to the polar component can be achieved by liquid adsorption chromatography (LAC) [11–13] on a normal-phase column or by liquid exclusion–adsorption chromatography (LEAC) [14,15] on a reversed-phase column. In the lower range of ethoxylation LEAC is superior to LAC, as it can be run in isocratic mode, while LAC typically requires gradient elution, with all the detection problems resulting therefrom [16–20].

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.

Numerous methods are mentioned in the literature, but none allows accurate quantitation as well as full resolution of the oligomers of the individual homologous series. Most of the established methods apply gradient elution, which requires evaporative light scattering detection (ELSD), thus makes quantitative determination of the lower oligomers impossible (methyl laurate and myristate are not detected by ELSD, and also the first two ethoxylates are severely underestimated). LEAC is run under isocratic conditions, which allows the use of refractive index (RI) detection, and thus accurate quantitation.

As we have shown in a previous communication [21], the transfer of fractions from LCCC to LEAC can be achieved using the full adsorption–desorption (FAD) technique: the fraction of interest is trapped (and focussed) on a short storage column by adding water to the eluate between the LCCC column and the FAD column. By this technique, all oligomers of lower fatty alcohol ethoxylates (FAEs) could be separated to the baseline.

In this paper, the new technique is now applied to fatty acid polyglycol esters.

2. Experimental

These investigations were performed using the density detection system DDS 70 (Chromtech, Graz, Austria), which has been developed in our group. Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS 70. Both columns and density cells were placed in a thermostatted box, in which a constant temperature of 25 °C was maintained for all measurements.

In LCCC, a flow rate of 0.5 ml/min was maintained with an ISCO 2350 HPLC pump (ISCO, Lincoln, NE, USA). A Zorbax 300 C_{18} column (150×4.6 mm, 3.5 μm, 300 Å) was used for all measurements. A Guard-Pak precolumn module (''Butterfly'') containing a μBondapak C_{18} cartridge (Waters, Milford, MA, USA) was used for focussing of fractions.

Samples were injected using a VICI injector (Valco Europe, Schenkon, Switzerland) with a 100-μl sample loop. A Sedex 45 ELSD apparatus (Sedere, Vitry sur Saine, France) was connected to the DDS 70. Nitrogen was used as the carrier gas, the pressure at the nebulizer was set to 1.0 bar and the temperature of the evaporator was set to 30 °C.

In LEAC, the mobile phase was delivered by a Jasco 880 PU pump (Japan Spectroscopic, Tokyo, Japan) at a flow rate of 0.5 ml/min. A Prodigy 5 μm ODS(3) column (250×4.6 mm, 5 μm, pore diameter 100 Å, series 185970, from Phemonenex, Torrance, CA, USA) was used in all measurements. A Bischoff 8110 RI detector (Bischoff, Leonberg, Germany) was connected to the DDS 70.

Both dimensions were coupled using a six-port two-position valve with an electric actuator (EC6W, from Valco Europe, Schenkon, Switzerland). Fig. 1 shows the experimental set-up.

The solvents (acetone, methanol and water, all HPLC grade) were purchased from Roth, Karlsruhe, Germany). The mobile phase compositions are always given in % (w/w) (i.e. 85% methanol denotes methanol–water, 85:15, w/w). The following polydisperse samples were used in these investigations (specifications given by the producer): polyethylene glycol monolaurate, average molecular masses 400 and 600, respectively (Sigma–Aldrich, Vienna, Austria), and Brij 30 (polyethylene glycol dodecyl ether), main component: tetraethylene glycol dodecyl ether (Fluka, Buchs, Switzerland). Fatty acid methyl ester ethoxylates (FAMEEs) were provided by W. Hreczuch, ICSO, Kedzierzyn-Kozle, Poland.

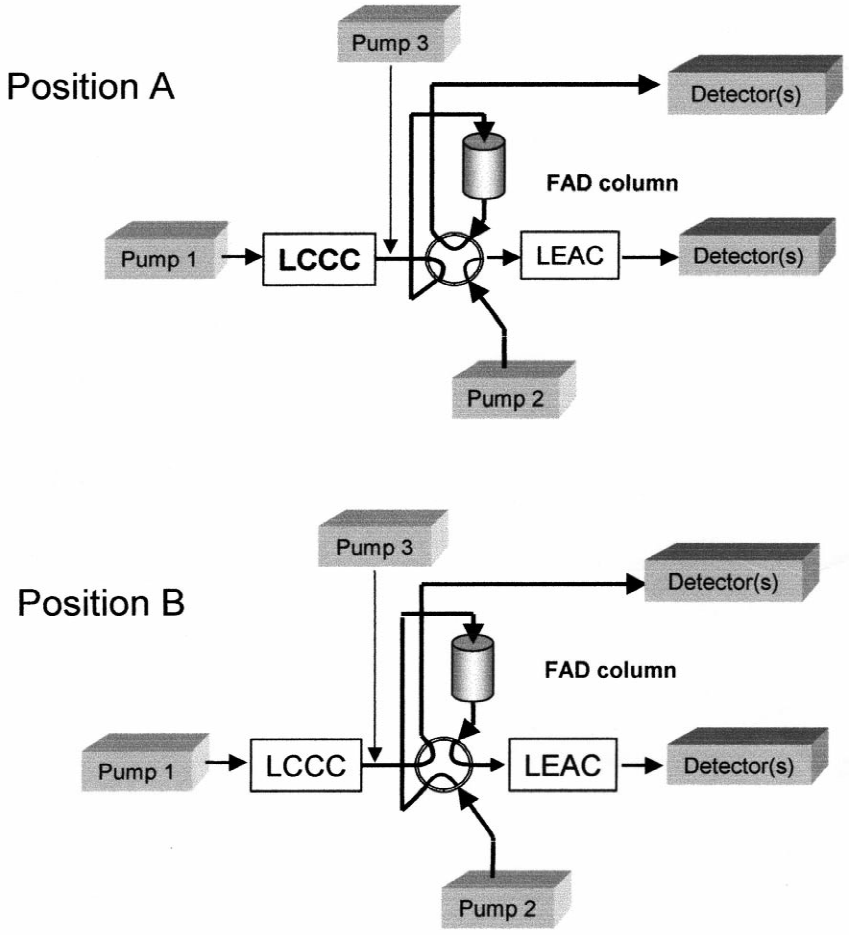

Fig. 1. Experimental set-up for two-dimensional LC with a combination of LCCC as the first and LEAC as the second dimension and FAD for focussing of fractions.

Monodisperse mono- and diesters of oligoethylene glycols were prepared by esterification of the fatty acid in toluene with *p*-toluenesulfonic acid as a catalyst [4].

3. Results and discussion

The samples were separated using the same approach described in the previous paper of this series [21], as is shown schematically in Fig. 1. The first dimension (LCCC) is linked to the second dimension (LEAC) by a six-port two-position valve. Additionally, the solvent stream passes through a short precolumn (FAD column), which is situated either behind the LCCC column (position A of the valve) or in front of the LEAC column (position B), as can be seen from Fig. 1a and b. Samples are injected with the valve in position A: on the LCCC column, separation according to functionality is achieved. As the fractions from LCCC are eluted in a volume which is too large, they must be focussed and reconcentrated before injecting them into the second dimension (LEAC) by switching the valve to position B.

Hence the fractions of interest are trapped on the FAD column by increasing the water content of the mobile phase after the LCCC column: when the peak of interest leaves the LCCC column, pump 3 adds water to the eluent to yield a mobile phase composition in which the fraction is completely adsorbed on the FAD column or even precipitated. The mobile

phase composition is controlled by the relative flow rates of pumps 1 and 3. Once the entire fraction is trapped on the FAD column, the valve is switched to position B, and the mobile phase used in LEAC rapidly desorbs the fraction, which is now injected onto the LEAC column as a much narrower zone than eluted from the LCCC column.

The performance of this approach is demonstrated in the following figures.

Fig. 2 shows a chromatogram of PEG monolaurate 400, which was separated by LCCC (in the first dimension) according to the length of the hydrophobic end group. Obviously this sample contains ethoxylates with C_{12} (main fraction) C_{14}, C_{16} and C_{18} end groups and some PEG in the solvent peak.

Fig. 3 shows another chromatogram of PEG 400 monolaurate, which was obtained under identical conditions as in Fig. 2, but the C_{12} fraction was completely cut out by switching on pump 3 shortly before the C_{12} fraction was supposed to appear. When the entire fraction had been trapped, pump 3 was switched off again, and the whole fraction was injected into the LEAC column by switching the six-port valve to position B. In the second dimension (LEAC) the individual oligomers of each homologous series are separated according to the number of oxyethylene units.

As can be seen from Fig. 4, the mobile phase composition has to be optimized for each fraction in order to achieve good separation of the ethoxylates. In the case of the C_{12} fraction of PEG monolaurate 400, a baseline separation is achieved in 60% of acetone, while the resolution is not perfect in 65%. On the other hand, the C_{16} fraction is perfectly resolved in 75% acetone (Fig. 5), while a mobile phase composition of 70% acetone mainly increases the analysis time. The C_{14} fraction of PEG monolaurate 400 is perfectly resolved in a mobile phase with 65% acetone (Fig. 6). For higher oligomers (as is the case in the C_{14} fraction of PEG monolaurate 600) the resolution is not that good in this mobile phase, but is still sufficient.

Figs. 7 and 8 show two-dimensional separations of two different fatty acid polyglycol ethers, PEG monolaurates 400 and 600. For both samples a mobile phase composition of 85% MeOH was used in the first dimension on the LCCC column. In Figs. 7 and 8 the second dimension represents the elution volume under the LEAC conditions, V_e(LEAC). It must be mentioned that these LEAC conditions were

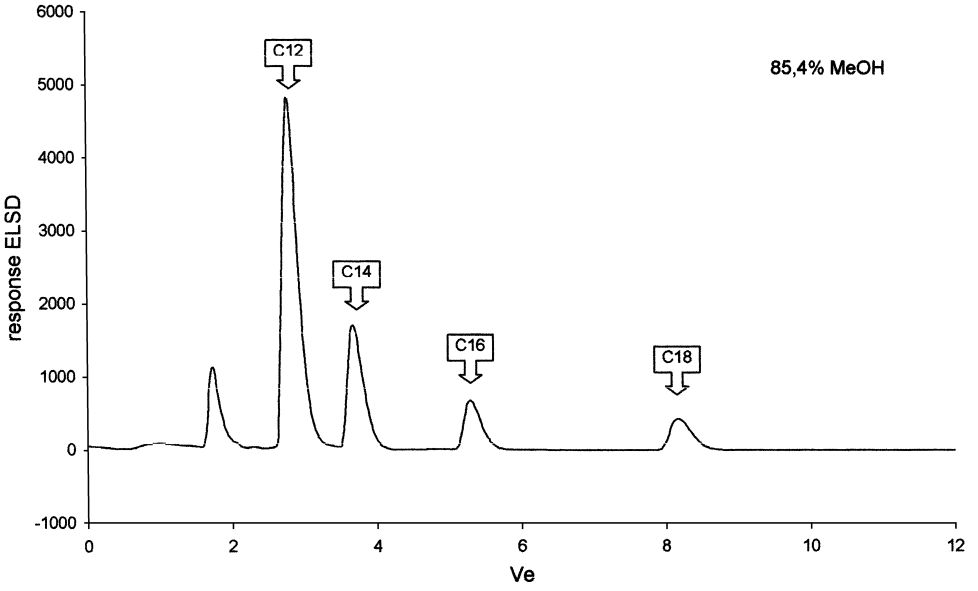

Fig. 2. LCCC of polyethylene glycol monolaurate 400 on Zorbax 300 C_{18} in methanol–water (85.4:14.6, w/w) with density and ELSD. V_e in ml.

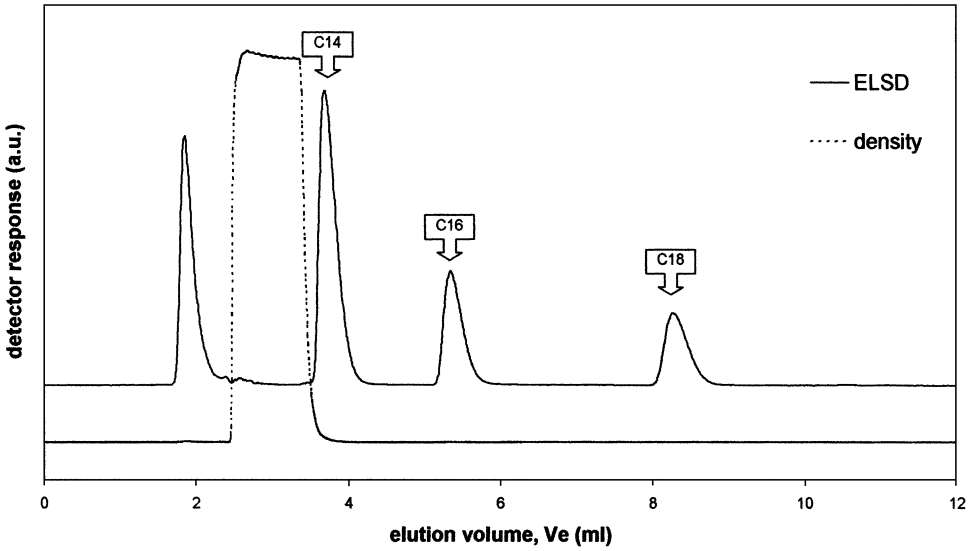

Fig. 3. LCCC of polyethylene glycol monolaurate 400 on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with density and ELSD. The C_{12} fraction has been removed from LCCC by the FAD technique and transferred to LEAC.

different for different fractions of fatty acid polyglycol ethers: the acetone content in acetone–water mobile phase was chosen to be ~60% for C_{12} fractions, 65% for C_{14}, 70% for C_{16}, and 75% for C_{18}. The LEAC calibration plots for these fractions and chosen conditions are shown in Fig. 9.

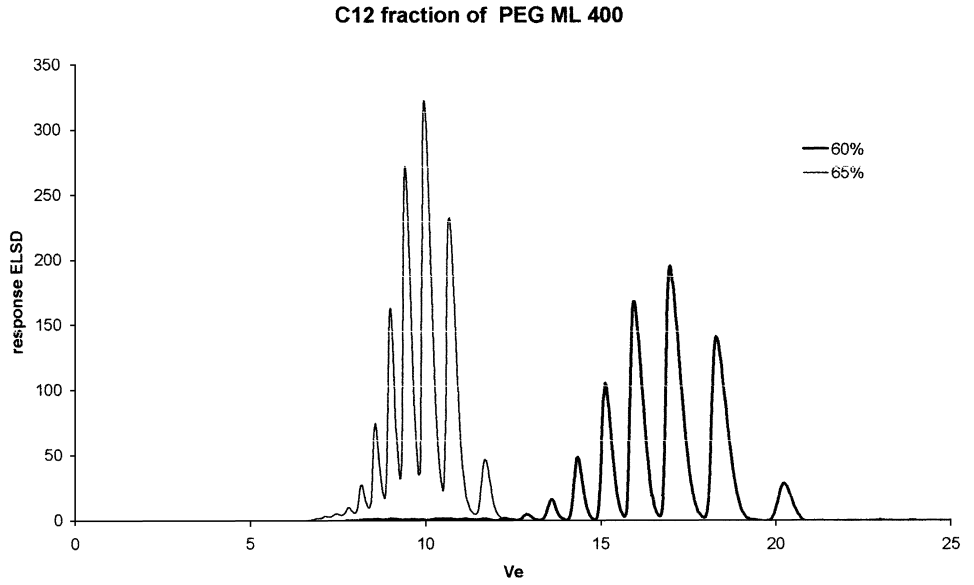

Fig. 4. LEAC of the C_{12} fraction from Fig. 3, as obtained on Prodigy ODS3 in acetone–water of different acetone content. Detection: RI. V_e in ml.

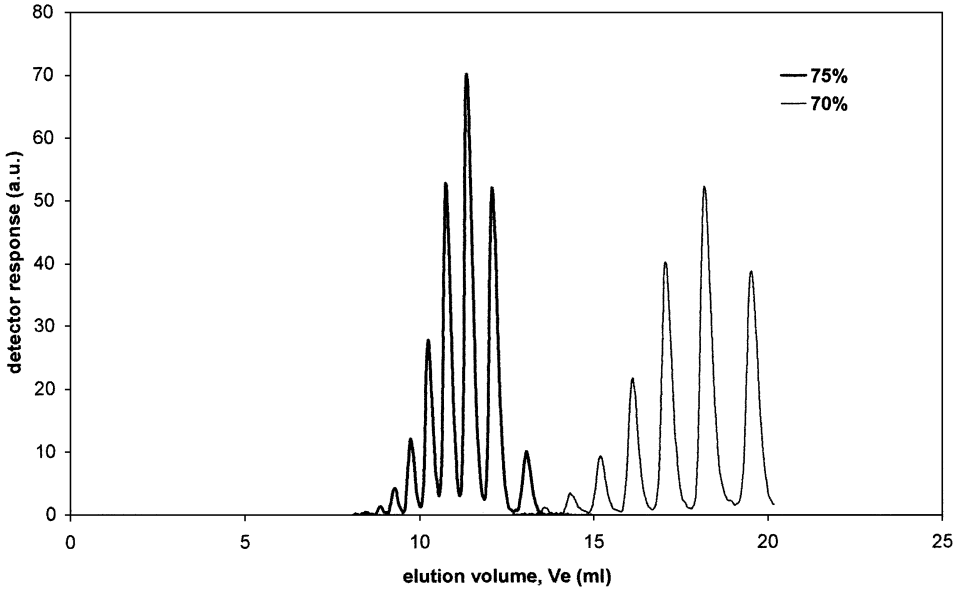

Fig. 5. LEAC of the C_{16} fraction of polyethylene glycol monolaurate 400, as obtained on Prodigy ODS3 in acetone–water of different acetone content. Detection: RI.

Using the calibrations of Fig. 9, it is possible to recalculate for each series the elution volume, V_e(LEAC), into the number of ethylene oxide (EO) units *n*, and to obtain the true two-dimensional distributions. In fact, as can be seen from Fig. 9, the LEAC conditions for different fractions were chosen so as to to provide for nearly the same dependence V_e(LEAC) versus *n*. This means that the second

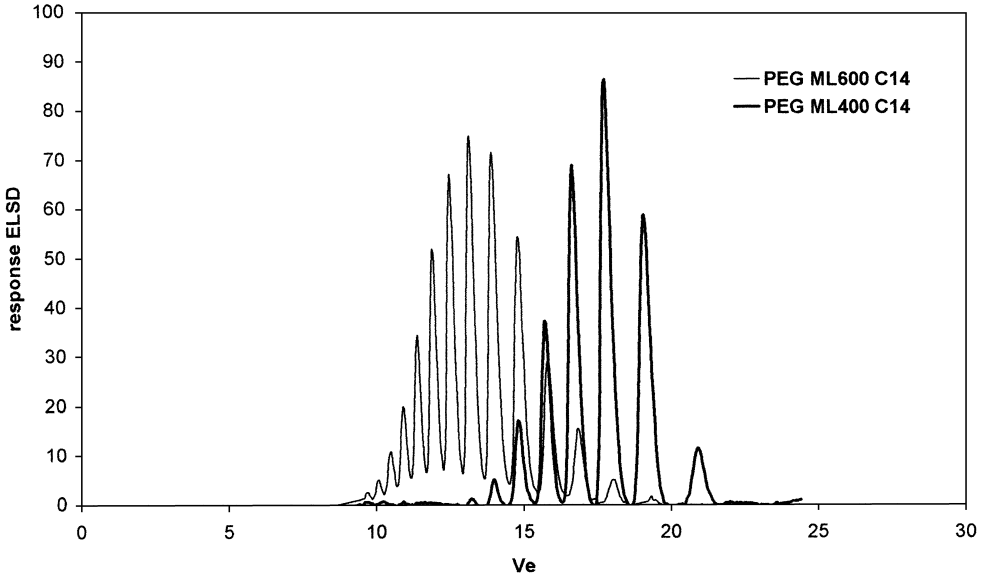

Fig. 6. LEAC of the C_{14} fractions of polyethylene glycol monolaurate 400 and polyethylene glycol monolaurate 600, as obtained on Prodigy ODS3 in acetone–water (71.1:28.9, w/w). Detection: RI. V_e in ml.

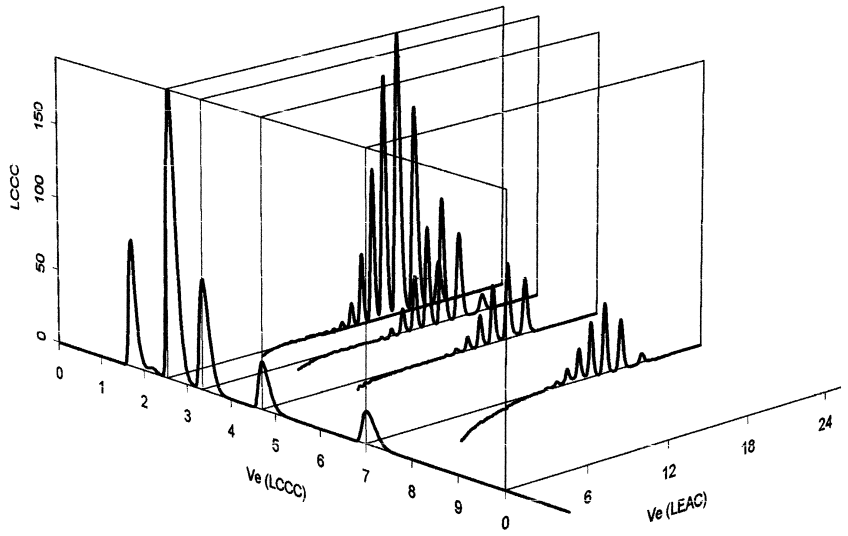

Fig. 7. Two-dimensional separation of polyethylene glycol monolaurate 400. First dimension (LCCC) on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with ELSD. Second dimension (LEAC) on Prodigy ODS3 in acetone–water of different acetone content (C_{12}: 60%, C_{14}: 65%, C_{16}: 70%, C_{18}: 75%). Detection: RI. V_e in ml.

dimension in Figs. 7 and 8, V_e(LEAC), represents in fact (in almost the same manner) the number of EO units in all fractions of fatty acid polyglycol ethers. Comparing Figs. 7 and 8, one can see that in both cases very well resolved chromatograms were obtained for each homologous series on the LEAC column by varying the acetone–water composition. As can be seen, this technique allows perfect sepa-

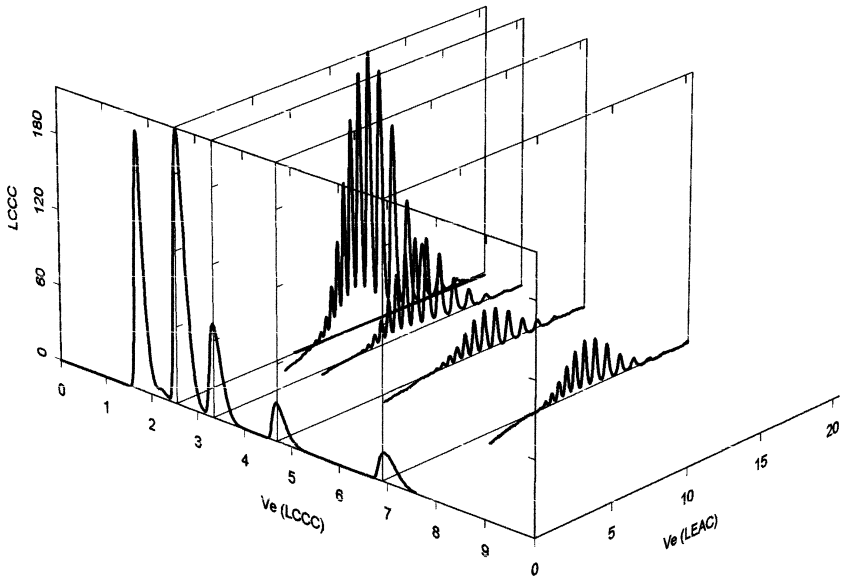

Fig. 8. Two-dimensional separation of polyethylene glycol monolaurate 600. First dimension (LCCC) on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with ELSD. Second dimension (LEAC) on Prodigy ODS3 in acetone–water of different acetone content (C_{12}: 60%, C_{14}: 65%, C_{16}: 70%, C_{18}: 75%). Detection: RI. V_e in ml.

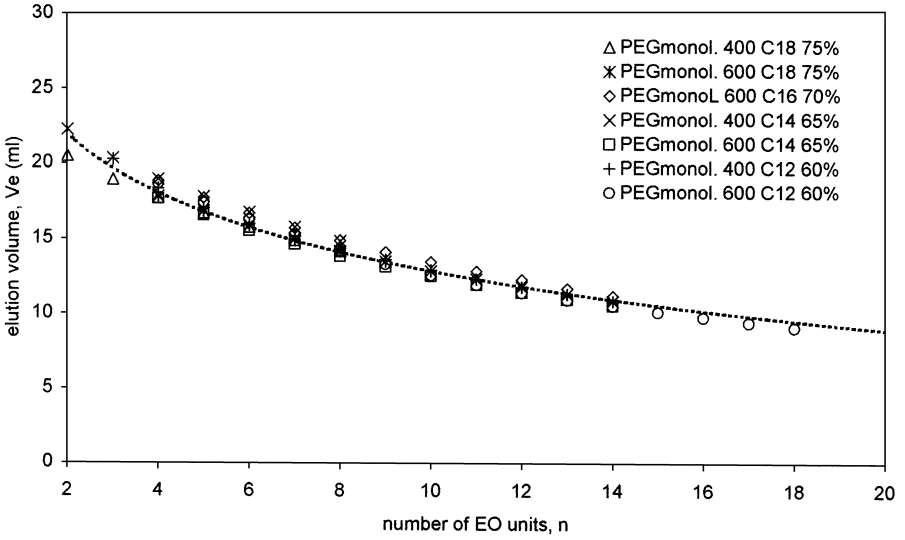

Fig. 9. LEAC calibrations for different fractions of fatty acid polyglycol ethers (PEG monolaurate 400, and PEG monolaurate 600). The acetone content in the acetone–water mobile phase for different fractions is given in the legend. Dotted line (given as a guide for eye) is an approximate representation of the entire data set.

ration of all monoester oligomers. Fatty acid polyglycol esters may, however, also contain the corresponding diesters. These should elute much later from the LCCC column. In the case of a sample based on C_{12}, C_{14}, C_{16}, and C_{18} acids, one may expect several series of diesters with symmetric and asymmetric substitution (C_{12}-PEG-C_{12}, C_{12}-PEG-C_{14}, C_{14}-PEG-C_{14}, and so on). Obviously, in the mobile phase corresponding to the CAP for PEG, only the C_{12} and C_{14} diesters will elute in a reasonable time. As can be seen from Fig. 10, there is indeed some material eluting at higher retention volumes in both samples.

When the ELSD sensitivity is increased after the monoesters peaks, it becomes obvious (Fig. 11), that the individual oligomers are at least partially resolved, although the chromatographic conditions were close to the conditions of LCCC. Spiking the sample with diethylene glycol dilaurate (Fig. 12) provides proof that the peak group eluting at V_e around 20 ml really belongs to C_{12}-PEG-C_{12} diester fraction. It is interesting that in the C_{12}-PEG-C_{12} series (and obviously also in the last visible series) the oligomers with low EO content elute later than those with high EO content. This specific type of LCCC behavior was discovered previously both experimentally [22] and theoretically [22,23] for difunctionals (or triblocks).

In recent papers [24,25] special software for simulation of chromatograms of polymers on the basis of the general theory of interactive chromatography has been described. The program uses a few parameters, which describe the column, the polymer to be analyzed and the interaction parameters. We extended this simulation software to deal with three-block copolymers and with a rough estimate of the required parameters simulated the expected chromatograms for the C_{12}-PEG-C_{12} and C_{12}-PEG-C_{14} diester fractions (Fig. 13). In both cases we used the same values of the interaction parameters for EO and CH_2 units, and the same molar mass distribution for central PEG block. As in the experiment, the enlarged peak calculated for the diethylene glycol dilaurate was specially added to the simulated chromatogram to show the position of this individual homologue in the chromatogram.

Comparison of the simulated chromatogram (Fig. 13) with the last two peak series in the real chromatogram (Fig. 12) shows a very similar pattern and even very similar positions of all individual peaks. This provides more proof that the two last series in the chromatograms of Figs. 11 and 12 really belong

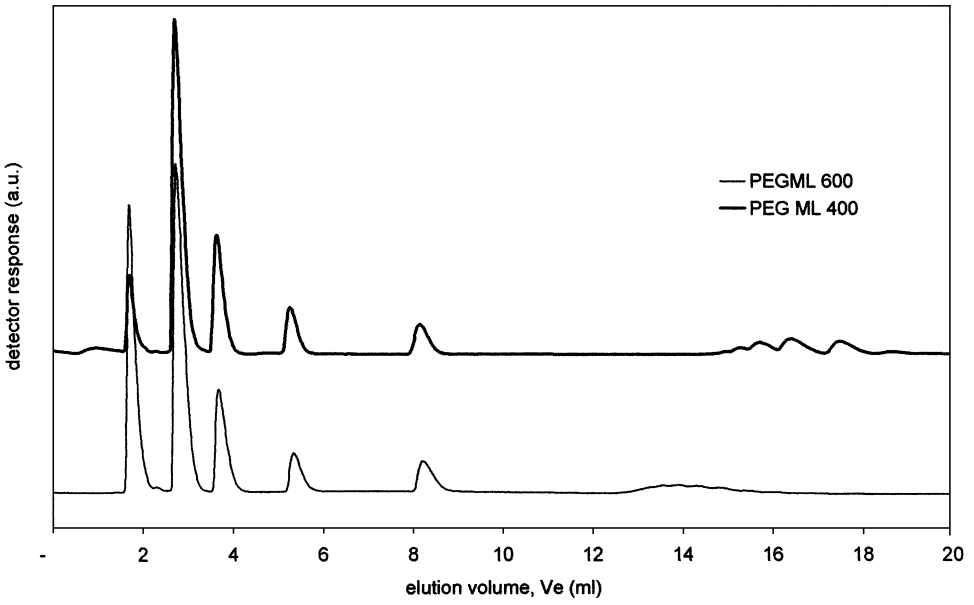

Fig. 10. LCCC of PEG dilaurate 400, as obtained on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with ELSD. Sensitivity: gain 4.

to the C_{12}-PEG-C_{12} and C_{12}-PEG-C_{14} diesters. In another communication we shall show that all series of diesters can be found in such samples under appropriate conditions.

4. Conclusions

Full separation of fatty acid polyglycol monoesters of up to 20 oxyethylene units can be achieved using

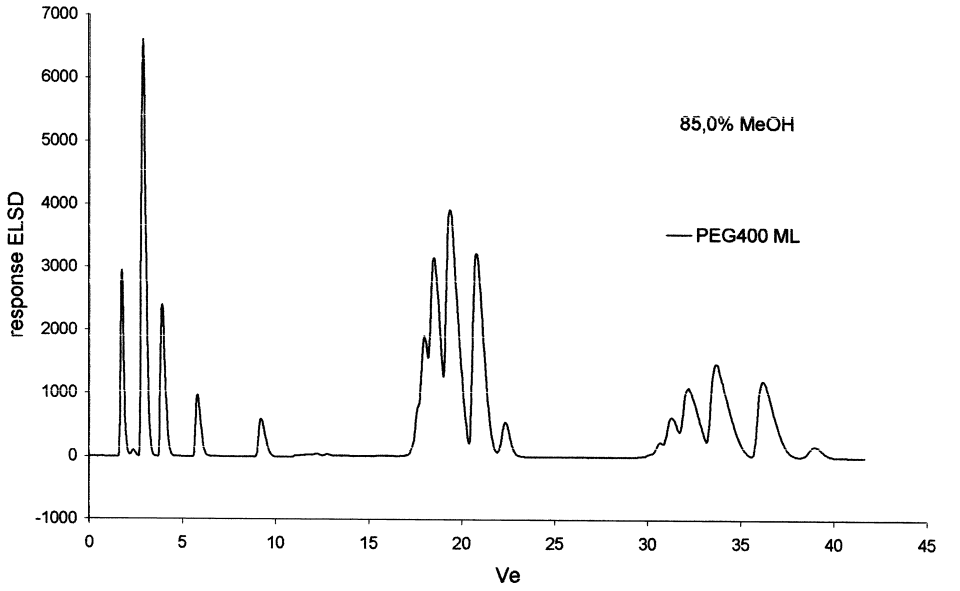

Fig. 11. LCCC of PEG dilaurate 400, as obtained on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with ELSD. Detector sensitivity: start gain 4, 1330 s: gain 6. V_e in ml.

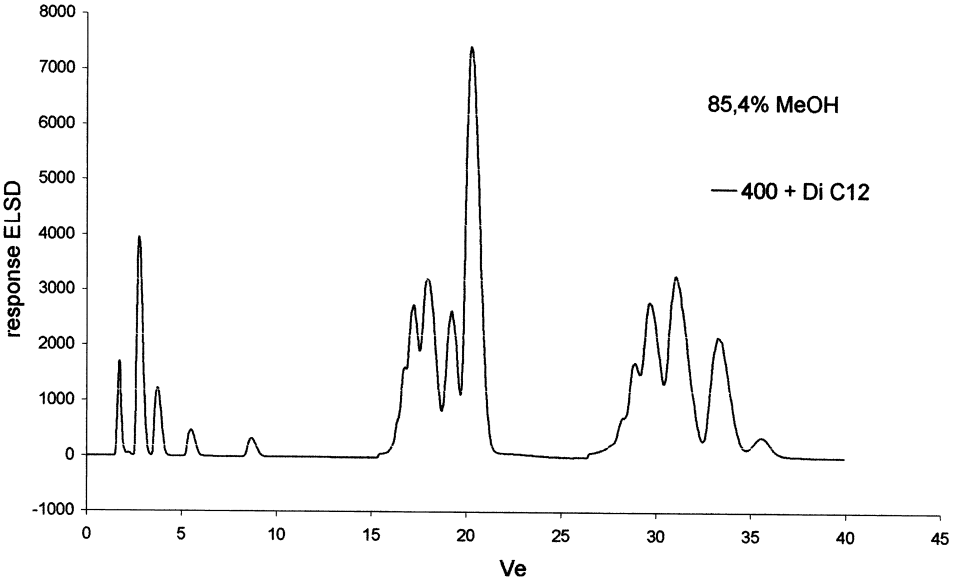

Fig. 12. LCCC of PEG dilaurate 400 spiked with di-EG dilauryl ester (C_{12}-EO_2-C_{12}), as obtained on Zorbax 300 C_{18} in methanol–water (85:15, w/w) with ELSD. Detector sensitivity: start gain 4; 1870 s: gain 6; 3190 s: gain 7. V_e in ml.

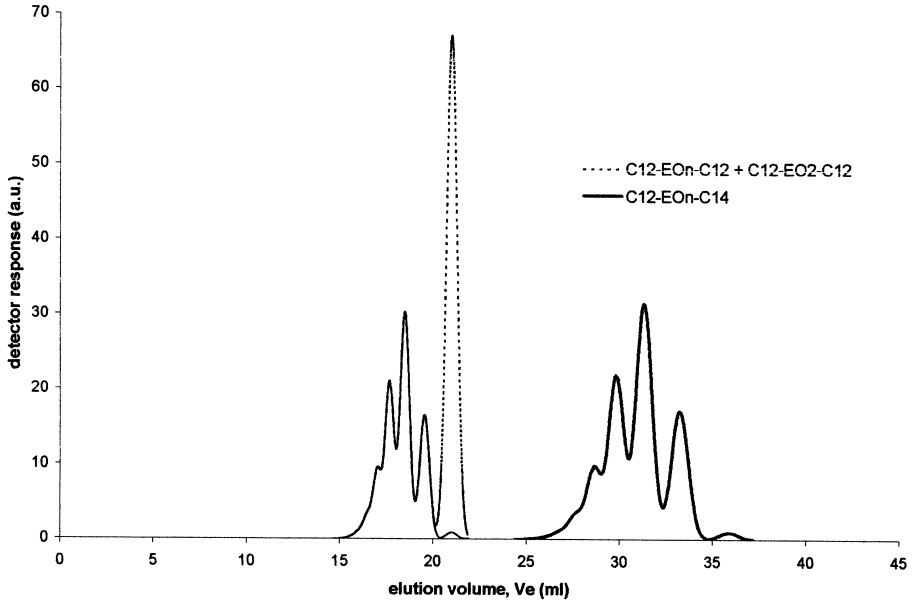

Fig. 13. Simulated chromatogram for the PEG 400-diesters C_{12}-EO_n-C_{12} (spiked with di-EG dilauryl ester C_{12}-EO_2-C_{12}) and C_{12}-EO_n-C_{14}. Parameters of simulation: pore radius 20.9 nm, void volume 1.72 ml, pore volume 0.73 ml, efficiency 5000, logarithmically normal molar mass distribution of PEG block with weight-average molecular mass (M_w)=187, and M_w/number-average molecular mass (M_n)=1.07, interaction parameter for EO unit 0.16 nm^{-1}, interaction parameter for CH_2 unit 3.28 nm^{-1}.

two-dimensional liquid chromatography with LCCC as the first and LEAC as the second dimension. The transfer of LCCC fractions to LEAC using the FAD technique is less time-consuming than collecting and isolating fractions and more reliable, as it avoids errors in sample handling. As LEAC is run in isocratic mode, the RI detector can be applied, which allows accurate quantitation. Diester oligomers, which can also be present in such a sample, are partially separated previously by a different mechanism in the first dimension (LCCC).

Acknowledgements

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References

[1] K. Kosswig, in: K. Kosswig, H. Stache (Eds.), Die Tenside, Carl Hanser, Munich, 1993, p. 115.
[2] A.N. Wrigley, F.D. Smith, A.J. Stirton, J. Am. Oil Chem. Soc. 36 (1959) 34.
[3] A.N. Wrigley, F.D. Smith, A.J. Stirton, J. Am. Oil Chem. Soc. 34 (1957) 39.
[4] J.L. Lewis, in: N.M. van Os (Ed.), Non-Ionic Surfactants—Organic Chemistry, Marcel Dekker, New York, 1998, p. 201.
[5] H. Pasch, I. Zammert, J. Liq. Chromatogr. 17 (1994) 3091.
[6] R.P. Kruger, H. Much, G. Schulz, J. Liq. Chromatogr. 17 (1994) 3069.
[7] D. Hunkeler, T. Macko, D. Berek, ACS Symp. Ser. 521 (1993) 90.
[8] A.V. Gorshkov, H. Much, H. Becker, H. Pasch, V.V. Evreinov, S.G. Entelis, J. Chromatogr. 523 (1990) 91.
[9] A.M. Skvortsov, A.A. Gorbunov, J. Chromatogr. 507 (1990) 487.
[10] A.M. Skvortsov, A.A. Gorbunov, D. Berek, B. Trathnigg, Polymer 39 (1998) 423.
[11] K. Rissler, H.P. Kunzi, H.J. Grether, J. Chromatogr. 635 (1993) 89.
[12] K. Rissler, U. Fuchslueger, H.J. Grether, J. Liq. Chromatogr. 17 (1994) 3109.
[13] N. Marquez, R.E. Anton, A. Usubillaga, J.L. Salager, J. Liq. Chromatogr. 17 (1994) 1147.
[14] B. Trathnigg, A. Gorbunov, J. Chromatogr. A 910 (2001) 207.
[15] B. Trathnigg, J. Chromatogr. A 915 (2001) 155.
[16] A.I. Hopia, V.M. Ollilainen, J. Liq. Chromatogr. 16 (1993) 2469.
[17] W. Miszkiewicz, J. Szymanowski, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1013.
[18] W. Miszkiewicz, J. Szymanowski, Crit. Rev. Anal. Chem. 25 (1996) 203.
[19] B. Trathnigg, M. Kollroser, D. Berek, M. Janco, Abstr. Pap. Am. Chem. Soc. 214 (1997) 220.
[20] B. Trathnigg, M. Kollroser, D. Berek, S. Nguyen, D. Hunkeler, in: T. Provder (Ed.), Chromatography of Polymers: Hyphenated and Multidimensional Techniques, American Chemical Society, Washington, DC, 1999, p. 95.
[21] B. Trathnigg, C. Rappel, J. Chromatogr. A 952 (2002) 149.
[22] A. Gorbunov, B. Trathnigg, J. Chromatogr. A, (in press).
[23] A.A. Gorbunov, A.M. Skvortsov, Vysokomol. Soed. A 26 (1984) 946.
[24] B. Trathnigg, A.A. Gorbunov, J. Chromatogr. A 910 (2001) 207.
[25] B. Trathnigg, A.A. Gorbunov, A.M. Skvortsov, J. Chromatogr. A 890 (2000) 195.