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Journal of Chromatography A, 1038 (2004) 43–52
JOURNAL OF CHROMATOGRAPHY A
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Thermodynamic study of retention in liquid exclusion–adsorption chromatography

Bernd Trathnigg*, Sandra Fraydl, Martin Veronik

Institute of Chemistry, Karl-Franzens-University, Heinrichstrasse 28, A-8010 Graz, Austria

Received 20 January 2004; received in revised form 10 March 2004; accepted 11 March 2004

Available online 14 April 2004

Abstract

The retention behaviour of fatty alcohol ethoxylates and fatty acid methyl ester ethoxylates on various reversed-phase columns in acetone–water has been studied in the regime of liquid exclusion–adsorption chromatography at different temperatures. Straight lines were obtained in the van't Hoff plots. The entropy and enthalpy changes were found to be negative (at least in the range of lower oligomers) and showed a dependence of the number of oxyethylene units. For higher oligomers, both entropy and enthalpy changes approach a constant value. This can be explained by the existence of a rather thick layer of organic solvent close to the surface of the stationary phase.

Keywords: Thermodynamic parameters; Liquid exclusion–adsorption chromatography; Retention mechanism; Temperature effects; Ethoxylates; Fatty alcohol ethoxylates; Fatty acid methyl ester ethoxylates; Alcohol ethoxylates

1. Introduction

Fatty alcohol ethoxylates (FAEs) are important products, which are produced in large amounts. According to the hydrophilic nature of the polyoxyethylene chain, they are used as nonionic surfactants or emulsifiers. Fatty acid methyl ester ethoxylates (FAMEEs) [1,2] are obtained by direct insertion of ethylene oxide (EO) to fatty acid methyl esters (FAMEs), which are well known as Biodiesel. FAMEEs and FAEs have similar surface tension-lowering characteristics [3,4].

These products often consist of different polymer homologous series (based on the purity of the fatty alcohols used as starting material). In this case their full characterization requires the independent determination of two distributions: molar mass (MMD) and type of functionality (FTD). This requires a two-dimensional separation, which can be achieved by combining different (chromatographic) techniques yielding complementary information: MMD or degree of ethoxylation and functionality (nature of hydrophobic end groups). An overview on the available techniques, their scope and limitations has been given in a recent paper [5].

(i) A separation according to the hydrophobic part can easily be achieved using liquid chromatography under critical conditions (LCCC) [6–11] at the critical adsorption point (CAP) for the EO unit on a reversed phase column.
(ii) A separation according to the degree of ethoxylation is often performed by liquid adsorption chromatography (LAC) on a normal phase column [12–14]. This typically requires gradient elution, which causes problems with detection. As FAEs do not contain chromophoric groups, there are different approaches [15], which are equally problematic: evaporative light scattering detection (ELSD) [16–20] underestimates the lowest oligomers or does not detect them at all. Derivatization introducing UV-absorbing [21–23] or fluorescent end groups [24,25] complicates the separation problem even more.

In previous communications [26,27], we have shown, that liquid exclusion–adsorption chromatography (LEAC) can overcome all these problems: this technique is run under isocratic conditions, thus it can be performed with refractometric (RI) detection, which allows an accurate quantitation, also in two-dimensional separations [28–30].

LEAC is performed on reversed phase columns in a mobile phase, in which the hydrophobic unit is rather strongly

* Corresponding author. Tel.: +43-3163805328; fax: +43-3163809840.
E-mail address: bernd.trathnigg@uni-graz.at (B. Trathnigg).

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doi:10.1016/j.chroma.2004.03.029

adsorbed, while the polyoxyethylene chain is eluted in the regime of size-exclusion chromatography (SEC). Consequently, the individual oligomers elute in SEC order, but far behind the void volume, as their hydrophobic end group is strongly adsorbed.

In an appropriate mobile phase, up to 20 oligomers can be resolved to the baseline. On most C_{18} columns this is the case in acetone–water mixtures containing 60–80% (w/w) acetone. Even samples containing more than one homologous series can be separated from each other, if their MMD is not too wide.

Similar separations have also been published by other authors [31–33], but typically in acetonitrile–water. Recently, a thermodynamic study has been published [34], which shows a rather strange behaviour of FAEs in acetonitrile–water.

We have now performed similar investigations in acetone–water mobile phases, which should give a deeper insight on the interaction process in LEAC.

2. Retention of homologous series in different modes of chromatography

The retention in liquid adsorption chromatography (LAC) is typically given in terms of the (dimensionless) retention factor k:

$$k = \frac{V_e - V_0}{V_0} \tag{1}$$

wherein V_e is the elution volume of a peak, and V_0 the void volume, which is generally considered as the elution volume of the solvent peak.

Obviously, k does not make sense in SEC, where all peaks elute before the void volume ($V_e < V_0$, and thus $k < 0$)!

In SEC, elution volumes are given by $V_e = V_i + KV_p$, wherein V_i is the interstitial volume (the volume between the particles of the packing), V_p the pore volume, and K the distribution coefficient between these volumes:

$$K = \frac{V_e - V_i}{V_p} = \frac{V_e - V_i}{V_0 - V_i} \tag{2}$$

Large molecules (with $K = 0$) elute at V_i (exclusion limit!), while small molecules, which do not interact with the stationary phase, should elute at the void volume $V_0 = V_i + V_p$ (as $K = 1$).

While retention factors have to be corrected for extra-column volumes (capillaries etc.), this is not the case for the distribution coefficients, as becomes clear from Eq. (2).

For the sake of convenience, most chromatographers using LAC prefer the retention factor over the distribution coefficient, as the latter requires the determination of interstitial volume and pore volume, which is typically performed in the same way as a SEC calibration using standards with known molar mass [35–40].

There is, however, still the problem of the correct definition (and determination) of the void volume, which is not trivial [41–48].

Basically, there are two different definitions [49]: the thermodynamic dead volume, which corresponds to V_0, and the kinetic dead volume, which corresponds to V_i. In other words, the volume of the mobile phase can be considered as "the total volume of eluent in the column" or "the elution volume of an unretained peak". The latter is not identical to the convention "nothing is adsorbed": if this peak contains a high molecular substance, it elutes at V_i, while low molecular substances may (or may not) elute at V_0. The question is, how to make sure, that the molecule used as a marker is really not adsorbed.

In the literature, different approaches towards the determination of the void volume have been described [41–48]. One of them uses an empirical relation, which describes the retention of homologous series (Martin's rule) [50]:

$$\ln k' = A + Bn \tag{3}$$

wherein A and B are constants for each system. Extrapolation to $n = 0$ should yield V_0. As has been shown in a previous paper [51], this is problematic, as Eq. (3) holds only in the range of strong interaction, i.e. at sufficiently high n, and there are considerable deviations at lower n!

While in LAC the retention increases exponentially with the number of repeat units, the opposite order is found in LEAC. As follows from the theory [27], the retention of amphiphilic molecules AB, which are considerably smaller than the pore diameter of the stationary phase, at low molar mass of non-adsorbing block A is given by:

$$K_{AB} \approx K_B \left(1 - \frac{\sqrt{\pi}}{2} c_B R_A\right) = K_B \left(1 - \tilde{C}\sqrt{M_A}\right) \tag{4}$$

while at higher M_A scales as

$$K_{AB} \approx \frac{K_B}{\sqrt{\pi}} \frac{1}{R_A c_B} \tag{5}$$

wherein K_{AB} and K_B are the distribution coefficients of the entire molecule AB and that of the adsorbed block (or end group) B, c_B is the corresponding interaction parameter, R_A is the radius of gyration and M_A the molar mass of the block eluting in exclusion regime.

In other words, the smallest oligomer of a polymer homologous series appears at the highest elution volume, while the distribution coefficients of the others decrease with the square root of the molar mass of the excluded block A.

3. Thermodynamics of liquid chromatography

From the thermodynamical point of view, the distribution coefficient $K = \exp(-G/RT)$ is related to the change of the Gibbs energy G of the polymer chain when it transfers from the free volume V_i into the pore volume V_p. Consequently, the distribution coefficient is related to the corresponding

entropy and enthalpy changes:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT\ln K \quad (6)$$

wherein ΔG°, ΔH°, and ΔS° are the changes in free energy, enthalpy and entropy, R the gas constant, and T the absolute temperature.

The correct relation between distribution coefficient and thermodynamic parameters is then:

$$\ln K = -\frac{\Delta G^\circ}{RT} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (7)$$

In a plot of $\ln K$ versus $1/T$ (van't Hoff plot), one may calculate ΔH° from the slope and ΔS° from the intercept.

Most chromatographers [52–57] follow, however, a pragmatic definition, which requires the introduction of the phase ratio ϕ (the ratio between the volumes of the stationary phase V_{st} and the mobile phase V_m):

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi \quad (8)$$

$$\phi = \frac{V_{st}}{V_m} \quad (9)$$

As has already been mentioned, different definitions are used for the volume of the mobile phase; and there is even more confusion about the volume of the stationary phase.

Most authors consider V_{st} as the free volume between the hydrocarbon chains of the stationary phase, the determination of which is, however, experimentally difficult [58–62].

Nevertheless, Eq. (8) is used instead of Eq. (7) in most papers.

Often no straight lines are obtained in the van't Hoff plot [63], while in other systems good linearity is observed [34,56,64]. These deviations can have different reasons: either the thermodynamic parameters or the phase ratio [63] vary with temperature. Several authors have described a temperature dependence of the void volume [43,46], which must be expected to originate from changes in the pore volume, as the interstitial volume should not vary considerably with temperature.

A way out of this dilemma has been proposed by Chester and Coym [63]: in the difference of the retention factors for adjacent oligomers the phase ratio is eliminated, hence the partial molar enthalpy of transfer added to oligomer i with the addition of one more unit to the oligomeric chain is obtained from the slope.

$$\ln k_{i+1} - \ln k_i = \frac{-(\Delta H^\circ_{i+1} - \Delta H^\circ_i)}{RT} + \frac{-(\Delta S^\circ_{i+1} - \Delta S^\circ_i)}{R} \quad (10)$$

$$\text{Slope} = \frac{-(\Delta H^\circ_{i+1} - \Delta H^\circ_i)}{R} = \frac{-\Delta H^\circ_d}{R} \quad (11)$$

From the definitions given in Eqs. (1) and (2) it becomes clear [51], that Eq. (8) is only an approximation, because there is no direct proportionality between k and K:

$$k' \equiv \frac{V_e - V_0}{V_0} \neq K\phi \quad (12)$$

Consequently, the distribution coefficient K should be applied instead of the retention factor for all types of liquid chromatography.

Obviously, the correct values for the pore volume have to be used for each temperature, when K (instead of k) is used in the van't Hoff plots. The nature of the temperature dependence of the pore volume is, however, not clear, at least not over a sufficiently wide temperature range. Consequently, the extrapolation from the quite narrow temperature range applicable in HPLC to $1/T = 0$ is questionable, as small variations of the pore volume (or the phase ratio) will strongly influence the absolute values of ΔH and ΔS.

Hence we have chosen a different approach: within the range used in this study, a constant value for V_i and a linear relation between V_p and T was assumed, and the entropic and enthalpic contributions were determined just for a single temperature (e.g. 25 °C) by plotting the parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$ as a function of the number of repeat units.

4. Experimental

These investigations were performed using the density detection system DDS70 (CHROMTECH, Graz, Austria). Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS70.

The columns and density cells were placed in a thermostatted box, in which temperature (from 15.0 to 35.0 °C) was kept constant to 0.1 °C using a thermostat (Lauda RM6, Lauda-Königshofen, Germany).

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 Bischoff 8110 refractive index detector (Bischoff, Leonberg, Germany) was connected to the DDS 70. Columns were connected to two column selection valves (Rheodyne 7060, Rheodyne, Cotati, CA, USA). The following columns were used in this study.

(i) Prodigy ODS(3): silica-based octadecyl column, 250 mm × 4.6 mm; particle diameter = 5 μm; nominal pore size =100 Å, obtained from Phenomenex, Torrance, CA, USA.
(ii) Jordi Gel DVB 500 RP: 100% poly(divinylbenzene); 250 mm × 4.6 mm; particle diameter = 5 μm; nominal pore size = 500 Å, obtained from Jordi, Bellingham, MA, USA

All measurements were performed with HPLC-grade solvents and the mobile phases were mixed by mass and vacuum degassed. Mobile phase composition was controlled by density measurement using a DMA 60 density meter equipped with a measuring cell, type DMA 602M (A. Paar, Graz, Austria). The HPLC solvents (acetone and

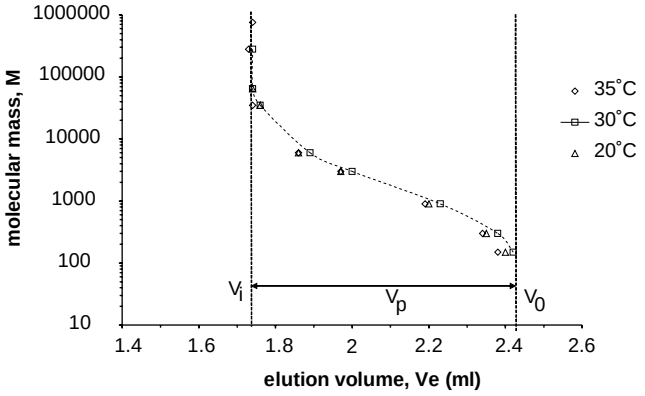

Fig. 1. SEC calibration for PEG on the Prodigy column in 65.98% acetone at different temperatures.

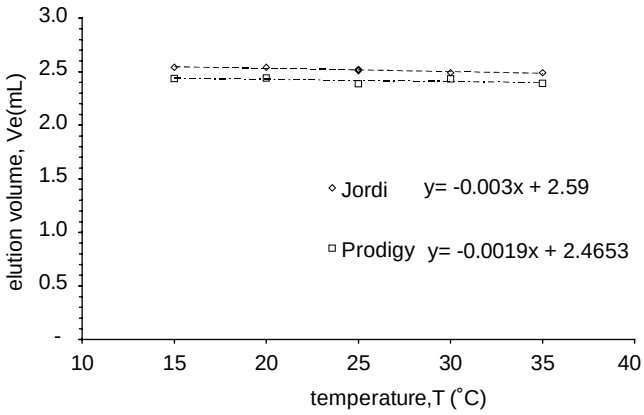

Fig. 2. Temperature dependence of the void volume of different columns in 65.98% (w/w) acetone.

water) were purchased from Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Polyethylene glycols were purchased from Sigma–Aldrich (Vienna, Austria). Fatty alcohol ethoxylates and fatty acid methyl ester ethoxylates were provided by "Blachownia" Institute of Heavy Organic Synthesis (ICSO), Kędzierzyn-Koźle, Poland.

5. Results and discussion

The first question concerns the determination of the interstitial and pore volume of the columns, which was performed by inverse SEC with polyethylene glycols.

Fig. 1 shows the results obtained for the Prodigy column in 65.98% (w/w) acetone at different temperatures. As can be seen, slightly different SEC calibration functions are obtained, in which the interstitial volume is fairly constant, the void volume (and thus the pore volume) depends on temperature (Fig. 2). This dependence is small, but should not be neglected.

In the subsequent measurements, the interstitial volume was considered to be constant, the void volume and the pore volume for each temperature was calculated using slope and intercept of the regression lines shown in Fig. 2.

Fig. 3 shows the LEAC separation of a fatty alcohol ethoxylate based on technical dodecanol (containing ~80% dodecanol and ~20% tetradecanol) with an average degree of ethoxylation $n_{EO} = 4$, which was produced at ICSO. As can be seen, two series of peaks are obtained, the first of

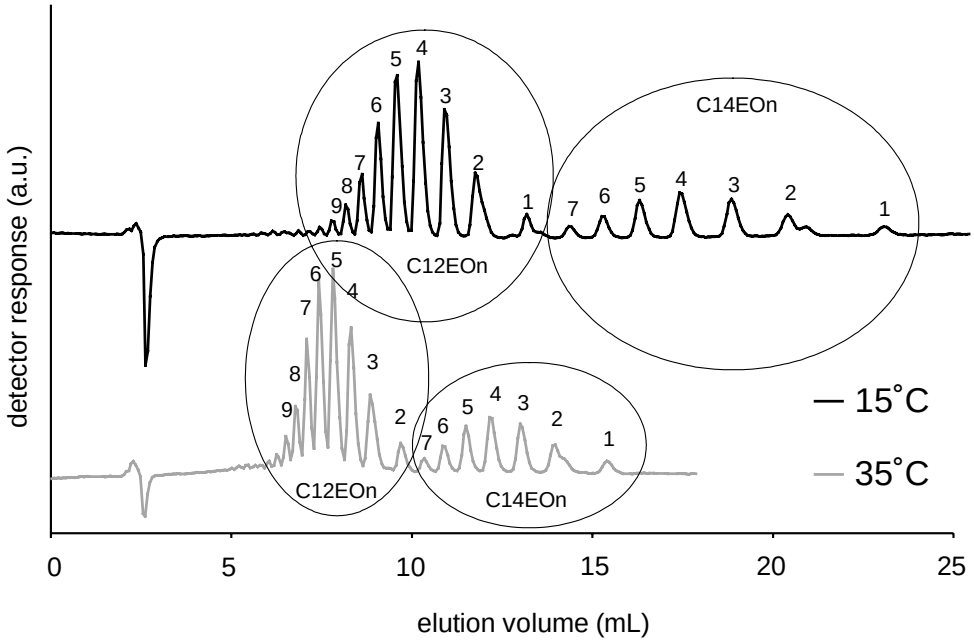

Fig. 3. LEAC of a of fatty alcohol ethoxylate based on technical dodecanol. Prodigy column, 70.91% acetone, temperature: 15.0 °C (top) and 35.0 °C (bottom).

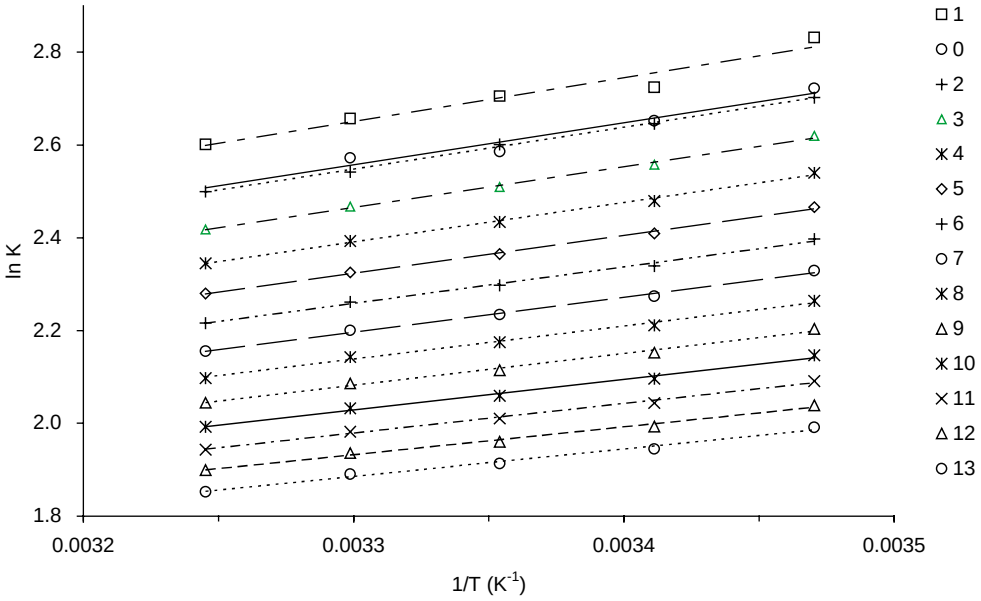

Fig. 4. van't Hoff plot of the C_{12} series of a fatty alcohol ethoxylate based on technical dodecanol. Prodigy column, 70.91% (w/w) acetone.

which represents the C_{12} series, and the second one the C_{14} series. The last peak within each series is the monoethoxylate, while the fatty alcohol (which is not present in this sample) would elute very close to the diethoxylate. The overall retention decreases with increasing temperature, while the resolution between and within the individual series does not change substantially.

Fig. 4 shows the van't Hoff plot for the C_{12} series: straight lines are obtained for the ethoxylate oligomers and the fatty alcohol (oligomer 0). The line of oligomers 0 and 2 almost coincide. A similar behaviour is observed for ethoxylates based on hexadecanol, which require a different mobile phase composition.

A similar separation can also be achieved on the Jordi column using a slightly different mobile phase composition. In the van't Hoff plot, straight lines are observed in this case, too (Fig. 5).

As can be seen in Fig. 6, straight lines are also obtained, when retention factors are used instead of distribution coefficients. This shows, that the non-linear plots in Cho et al.'s paper [34] are due to a fundamental difference between acetonitrile and acetone as organic modifier!

Fig. 7 shows a LEAC separation of a mixture of three C_{16} ethoxylates with different (average) degree of ethoxylation (Brij 52, 56 and 58). In this chromatogram, peaks can be identified up to the oligomer 30. The corresponding van't Hoff plot is shown in Fig. 8. Again straight lines are obtained, and the fatty alcohol elutes earlier than the monoethoxylate, and close to the diethoxylate.

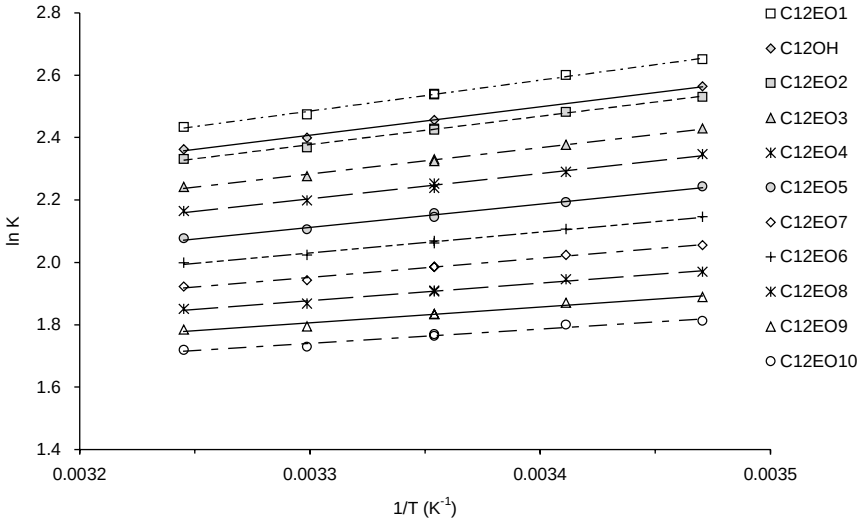

Fig. 5. van't Hoff plot of the C_{12} series of a fatty alcohol ethoxylate based on technical dodecanol. Jordi column, 65.98% (w/w) acetone.

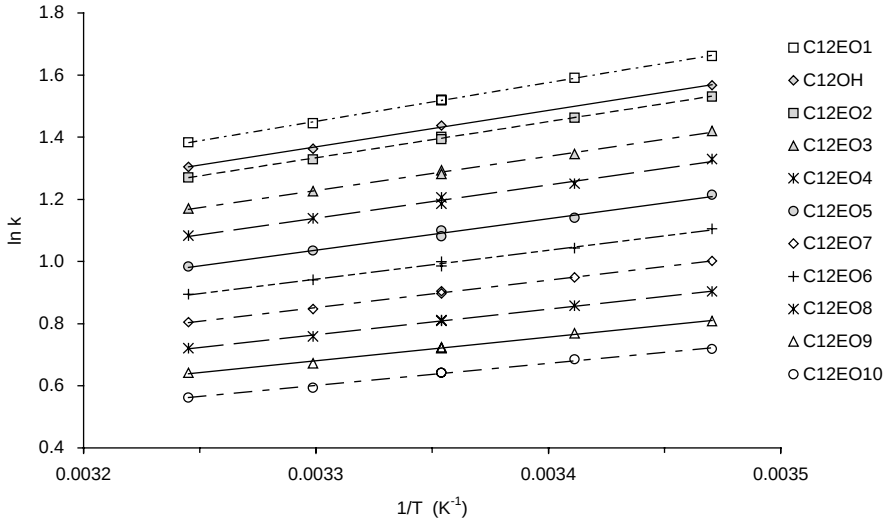

Fig. 6. Plot of $\ln k$ vs. $1/T$ from the same data set as in Fig. 5.

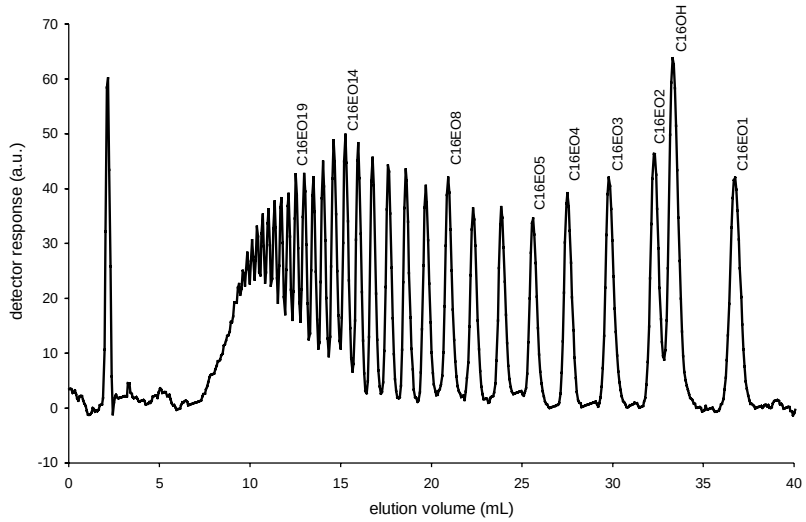

Fig. 7. LEAC of a mixture of fatty alcohol ethoxylates based on hexadecanol (Brij 52, 56, and 58). Prodigy column, 72.4% (w/w) acetone, 15.0 °C.

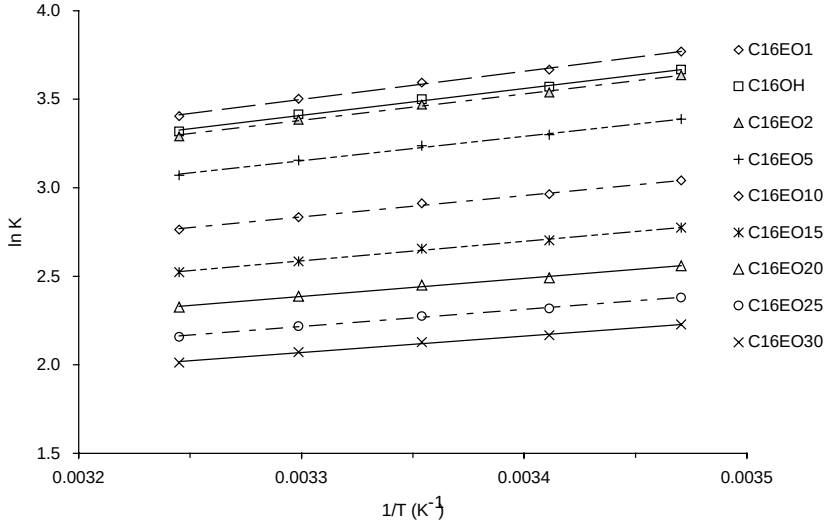

Fig. 8. van't Hoff plot of the C_{16} series of fatty alcohol ethoxylates. Prodigy column, 72.4% (w/w) acetone.

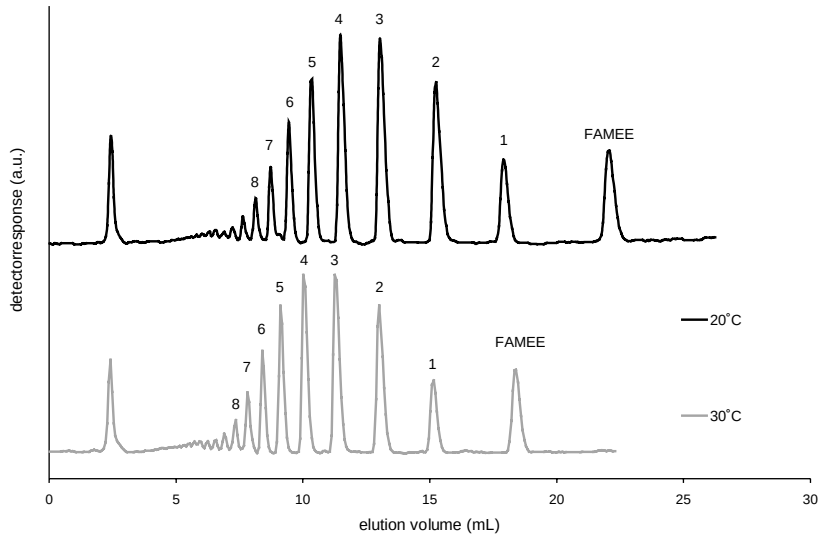

Fig. 9. LEAC of a FAMEE based on pure methyl dodecanoate. Prodigy column, 70.91% (w/w) acetone, temperature: 20.0 °C (top) and 30.0 °C (bottom).

As has already been pointed out [26,27], this at the first sight unexpected behaviour is due to the fact, that the terminal hydroxy group of the fatty alcohol pulls the last methylene group out of the adsorption layer, which leads to a decrease in retention.

The situation is somewhat different in the separation of ester ethoxylates: Fig. 9 shows the separation of a fatty acid methyl ester ethoxylate (FAMEE) based on pure dodecanoic acid with an average degree of ethoxylation $n_{EO} = 3$, which was produced at ICSO. The corresponding van't Hoff plot is shown in Fig. 10. As can be seen, straight lines are also obtained in this case, but the fatty acid methyl ester (FAME) elutes as the last peak, as should be expected. Obviously, the fatty ester, which does not contain a polar end group (as is the case in the fatty alcohol), has full access to the adsorption layer.

Fig. 11 shows a plot of the parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$, which were obtained for the C_{12} series of a FAE as a function of the number of repeat units.

In Fig. 12 the results for a C_{12} FAMEE are shown. As can be seen, retention is governed by enthalpy, but a small influence of entropy is also observed in both cases.

The results on the Jordi column (from the van't Hoff plot shown in Fig. 5) look very similar, as can be seen from Fig. 13. For comparison, the changes in entropy and enthalpy, ΔS and ΔH, respectively, were calculated for each series of oligomers from slope and intercept of the van't Hoff plots.

As can be seen from Fig. 14, ΔS is negative for the lower oligomers of all series on the Prodigy column, its absolute value increases from C_{12} to C_{16} and decreases with the number of EO units. The slope of this linear dependence is the

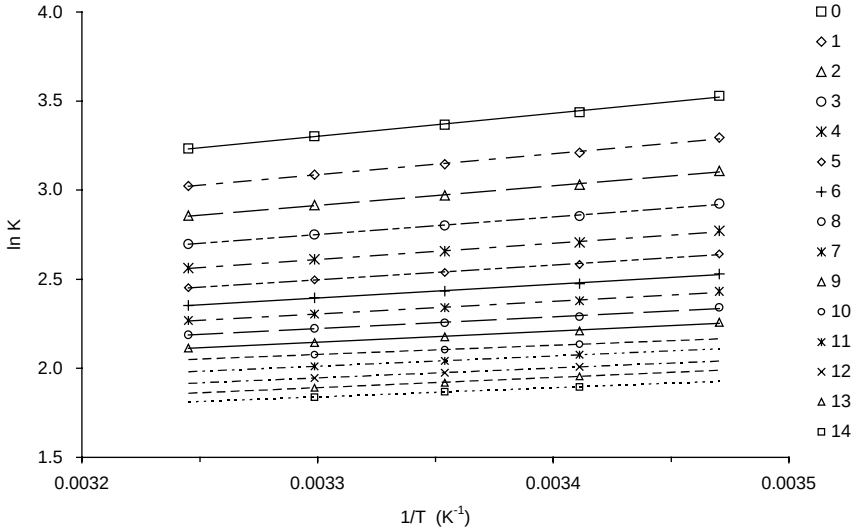

Fig. 10. van't Hoff plot of fatty acid methyl ester ethoxylates (C_{12} FAMEE). Prodigy column, 70.91% (w/w) acetone.

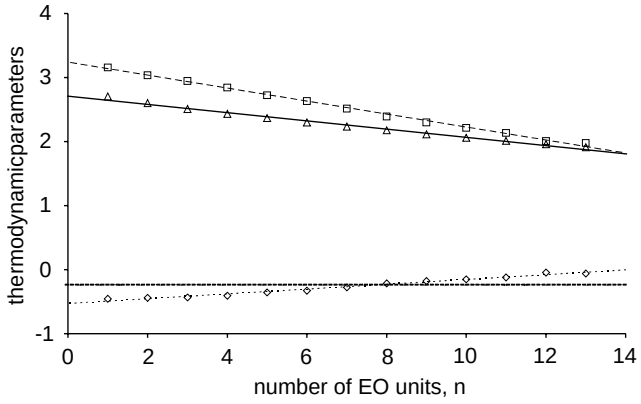

Fig. 11. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (□), and $\Delta S/R$ (◇) in LEAC of C_{12} FAE. Prodigy column, 70.91% (w/w) acetone.

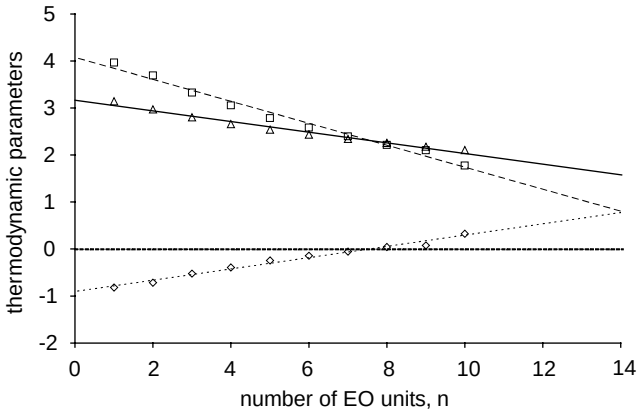

Fig. 12. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (□), and $\Delta S/R$ (◇) in LEAC of a C_{12} FAMEE. Prodigy column, 70.91% (w/w) acetone, temperature: 25.0 °C.

same for the FAE series, while it is considerably larger for the FAMEEs.

The dependence of ΔS on the number of EO units is reasonable, as PEG elutes in such a mobile phase composition in the SEC mode, which also means, that $\Delta S < 0$.

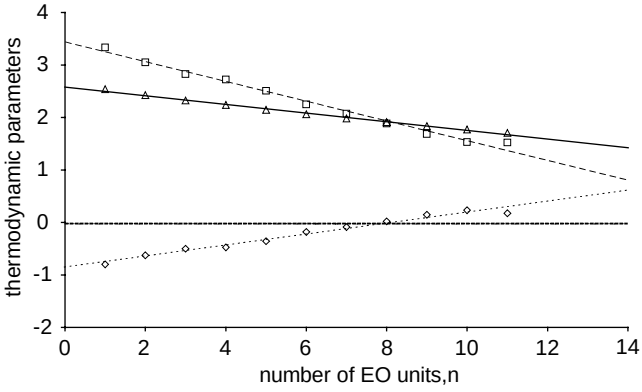

Fig. 13. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (□), and $\Delta S/R$ (◇) in LEAC of C_{12} FAE. Jordi column, 65.98% (w/w) acetone.

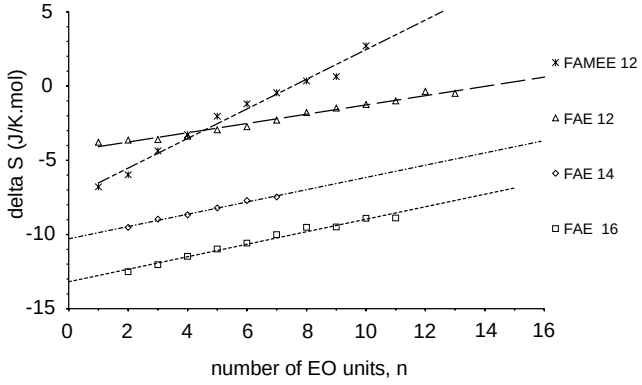

Fig. 14. Entropy changes in LEAC of different polymer homologous series. Prodigy, 70.91% (w/w) acetone.

A similar picture is found for ΔH (Fig. 15), which is negative for all oligomers, as expected. It is, however, remarkable, that ΔH depends on the number of EO units.

A possible explanation may be different partitioning of the individual oligomers into the layer of the stationary phase, which is responsible for adsorption.

From the data obtained with the Prodigy column it seems, however, that the relation of the thermodynamic parameters and the length of the polyoxyethylene chain is linear only for the lower oligomers.

When the measurement is extended to higher oligomers, a different behaviour is observed: Fig. 16 shows the thermodynamic parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$ as a function of the number of repeat units for the C_{16} series of fatty alcohol ethoxylates, which were obtained with a mixture of Brij 52, 56, and 58 on the Prodigy column in 72.4% acetone (see corresponding chromatogram on Fig. 7).

As can be seen from Figs. 17 and 18, ΔS and ΔH approach a nearly constant negative value on the Prodigy column for chains with more than 20 repeat units. The curvature cannot be seen clearly with the Jordi column, the separation range of which is not sufficient.

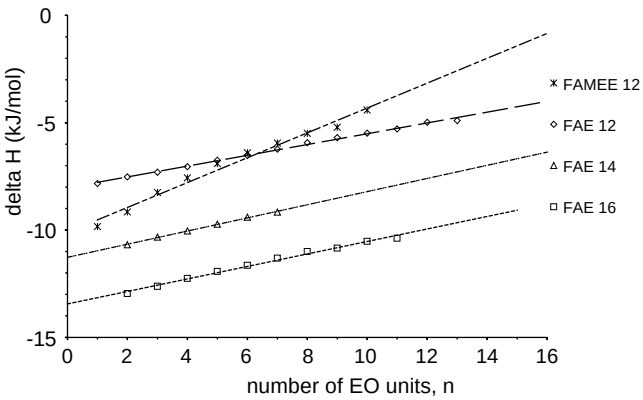

Fig. 15. Enthalpy changes in LEAC of different polymer homologous series. Prodigy, 70.91% (w/w) acetone.

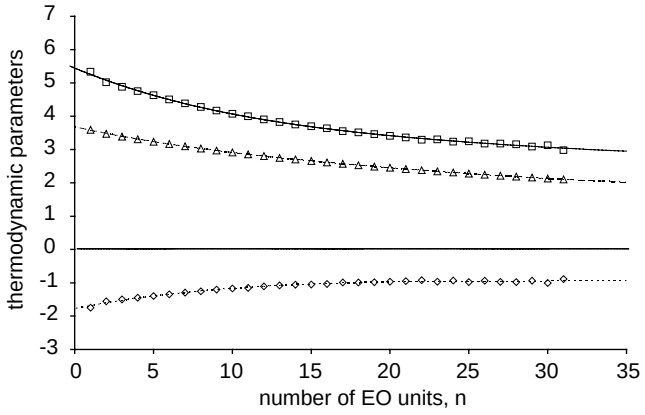

Fig. 16. Thermodynamic parameters $-\Delta G/RT$ (△), $-\Delta H/RT$ (□), and $\Delta S/R$ (◇) in LEAC of C_{16} FAE. Prodigy, 72.4% (w/w) acetone.

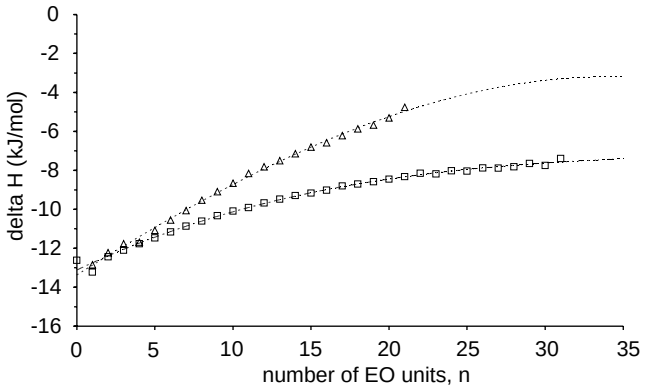

Fig. 17. Enthalpy changes in LEAC of a C_{16} fatty alcohol ethoxylate (Brij 52) in 72.4% (w/w) acetone. Columns: Prodigy (□) and Jordi (△).

This behaviour may be explained following the findings of Kazakevich et al. [65], who found strong evidence for a rather thick boundary layer of the pure organic solvent close to the surface of the stationary phase in mixed mobile phases containing water and acetonitrile or tetrahydrofuran (THF). This seems to hold true also in the case of acetone–water.

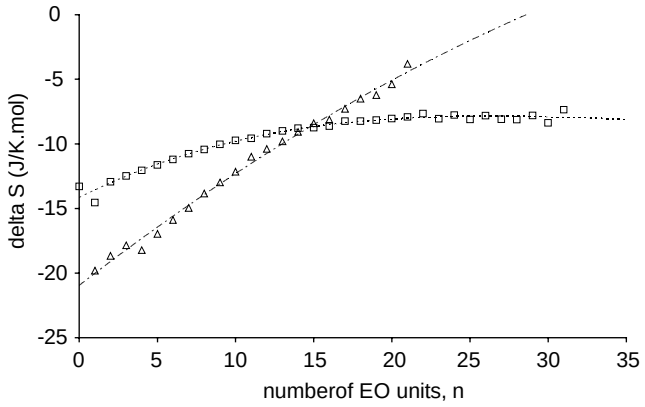

Fig. 18. Entropy changes in LEAC of a C_{16} fatty alcohol ethoxylate (Brij 52) in 72.4% (w/w) acetone. Columns: Prodigy (□) and Jordi (△).

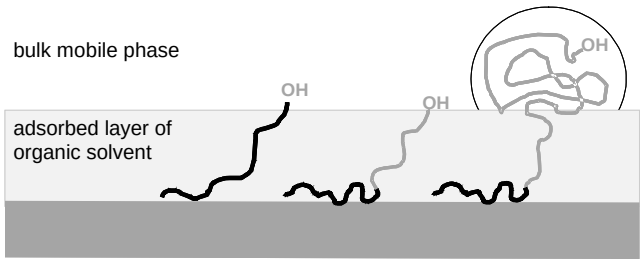

Fig. 19. Schematic representation of the possible structure of adsorbing amphiphilic molecules in LEAC (black line: hydrocarbon chain, grey line: EO chain).

If the hydrophobic moiety of an amphiphilic molecule (such as FAE, FAMEE etc.) is adsorbed on the surface of the stationary phase, it pulls at least a part of the hydrophilic chain segment into an thermodynamically less suitable environment (as indicated in Fig. 19).

This agrees quite well with the following findings.

(i) While FAMEs elute as the last member in the series of FAMEEs, the fatty alcohols (which contain a polar end group) are shifted towards lower elution volumes (close to the oligomer with two oxyethylene units).
(ii) ΔH is strongly negative for rather short EO chains. It becomes less negative with increasing number of EO units and approaches a constant (negative) value for longer EO chains.

6. Conclusions

The retention of FAEs and FAMEEs on reversed-phase columns in acetone–water under LEAC conditions is influenced by the end groups and the number of repeat units: it increases with the number of methylene groups in the hydrophobic part and decreases with increasing number of oxyethylene units in the hydrophilic part. The van't Hoff plots show straight lines, from which the thermodynamic parameters ΔS and ΔH can be calculated. On the columns used in this study, both parameters showed a dependence on the number of oxyethylene (EO) units.

This could be explained by the following model of a thick adsorbed layer of organic solvent, which is shown schematically in Fig. 19. Interactions are assumed highly favorable for the CH_2 groups and unfavorable for EO groups and for the hydroxy group. This explains the lower retention of the fatty alcohol. When the EO chain of n units is short, it may be completely pulled into the layer (maybe except for the terminal OH group), so one may expect the enthalpy change depending on n. If a copolymer has a long EO chain of total n EO units, this EO block will form a flower-like structure with a stretched stem of n^* EO units inside and a coiled crown of n–n^* units outside the organic layer. In this case n^* is expected to be a function of both interaction parameters and on the layer thickness and structure.

Acknowledgements

We would like to thank Dr. Alexei Gorbunov (Institute for Highly Pure Biopreparations, St. Petersburg, Russia) for many fruitful discussions.

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