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Retention mechanism of fatty alcohol ethoxylates in reversed-phase liquid chromatography

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Abstract

Fatty alcohol ethoxylates (FAEs) are widely used nonionic surfactants that have distributions in both alkyl and poly(ethylene oxide) (PEO) chain length. Generally, two-dimensional liquid chromatography technique is required for the complete characterization of both distributions. By selecting a proper stationary and mobile phase condition, however, we can obtain fully resolved chromatograms of a FAE sample (Brij 30) with respect to both alkyl and PEO chain length by using a single reversed-phase C_{18} column and aqueous acetonitrile mobile phase. FAEs show a peculiar reversed-phase liquid chromatography (RPLC) retention behavior with an aqueous–organic mobile phase, the retention mechanism of which has not been fully elucidated. For a fixed alkyl chain length, FAEs with higher-molecular-mass PEO block elutes first and the van't Hoff plot of the retention factor shows a curvature. The unique retention behavior can be understood from the opposite thermodynamic characteristics associated with RPLC retention of PEO block and alkyl chain: the sorption process of PEO to the non-polar stationary phase shows $\Delta H^{\circ}>0$ and $\Delta S^{\circ}>0$ while the alkyl chain shows $\Delta H^{\circ}<0$ and $\Delta S^{\circ}<0$ in contrast. The relative magnitude of the two contributions can change the elution order of the FAE. Therefore the often found, inverted elution order of FAEs (the early elution of FAEs with longer PEO block) is due to the positive enthalpic interaction of PEO blocks, which is a characteristic of the hydrophobic interaction. And the curvature of the van't Hoff plots was analyzed assuming the temperature dependent thermodynamic variables.

Keywords: Retention mechanism; Temperature effects; Fatty alcohol ethoxylates; Alcohol ethoxylates; Ethoxylates

1. Introduction

Fatty alcohol ethoxylates (FAEs) are an important class of non-ionic surfactants composed of a poly-(ethylene oxide) (PEO) block and an alkyl chain. They are commonly prepared by addition of ethylene oxide (EO) to long chain aliphatic alcohols under base-catalyzed conditions. Commercial products are complex mixtures of oligomer blocks with different numbers of EO units and often with different alkyl chain lengths of parent fatty alcohols. The molecular mass distribution of the individual blocks depends on the reaction conditions and on the molar ratio of the compounds in the reaction mixture. The distributions of both PEO and alkyl blocks and the ratio of hydrophobe to hydrophile affect the chemical and application properties. Thus, both PEO and alkyl

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distributions need to be characterized for performance and quality control purposes.

High-performance liquid chromatography (HPLC) has been most widely used to characterize FAE samples [1,2]. The PEO distribution can be analyzed easily by normal-phase LC (NPLC) on columns packed with unmodified silica gel or with amino-, nitrile- or diol-chemically bonded phases [3–9], while alkyl chain length distribution is commonly analyzed by reversed-phase LC (RPLC) with C_{18}- or C_8-bonded silica columns [9–18]. In most cases mixed aqueous organic mobile phase was employed. Combining RPLC and NPLC on-line, Murphy et al. demonstrated a simultaneous alkyl and PEO distribution analysis by two-dimensional (2D) LC [19]. Recently Trathnigg and coworkers also reported on the characterization of FAEs using 2DLC with liquid chromatography under critical conditions (LCCC) as the first and reversed-phase LC as the second dimension [20].

Despite the large volume of the successful analysis results reported so far, the separation mechanism of FAEs is not fully understood. There exist many reports on the peculiar retention behavior of FAE samples in both RPLC and NPLC systems. For example, Lemr et al. reported the effect of mobile phase composition on the retention of FAE derivatives in RPLC [12]. They found that the elution order was inverted with the composition of the aqueous acetonitrile mobile phase. At low acetonitrile content, FAEs with a large number of EO units eluted first. As the acetonitrile content increases, the elution order was inverted and FAE of a small number of EO units eluted first. In between, the co-elution of FAEs independent of PEO chain length occurred. Jandera et al. also reported on the peculiar retention behavior of FAE and explained the retention behavior in terms of the combined effect of the polarity of the mobile phase and the solvation of EO groups, which depends on the water content in the aqueous mobile phase [9].

Recently, Trathnigg and Gorbunov proposed a rather new mechanism, exclusion-adsorption chromatography for the RPLC separation of the nonionic surfactant [17,18,21]. They suggested that in the isocratic RPLC separation, FAEs of a fixed alkyl chain length are separated according to the number of EO units by two concerted mechanisms, interaction of FAEs with the stationary phase and the size exclusion from the pore. Adjusting the parameters associated with the two separation mechanisms, they showed that the simulated chromatogram mimicked the real chromatogram quite well. Also it successfully explains the peculiar RPLC retention behavior of FAEs that high-molecular-mass FAEs (larger number of EO units) elutes first. According to the model, the attractive interaction between FAEs and the stationary phase yields the positive retention factor while the size exclusion mechanism is responsible for the inverted elution order.

However, it is not necessary to attribute the inverted elution order to the size-exclusion mechanism. For an example, Lochmüller et al. found such an inverted elution order in RPLC separation of homo-PEO samples [22]. The water-soluble polymer shows the entropy–enthalpy compensation point at far from the total void volume of the column, which indicates that the co-elution behavior is not a result of compensation between the size-exclusion and the interaction mechanism commonly found in many other organic polymers [23–26]. At a fixed acetonitrile composition of the aqueous mobile phase, the elution order changes with temperature. At high temperature, the normal elution order was observed, i.e. low-molecular-mass PEO elutes first. As the temperature is lowered, it passes a co-elution point and high-molecular-mass PEO begins to elute first analogous to the elution order found in the size-exclusion chromatography. However, the separation mechanism is not a size exclusion process since the polymers elute after the total void volume of the column and the retention strongly depends on temperature, which are not expected in the entropy-driven size-exclusion process.

The temperature dependence of the RPLC retention of PEO is quite different from the other organic polymers: the retention increases as the temperature increases. The sorption process of PEO to the non-polar stationary phase showed $\Delta H^{\circ}>0$ and $\Delta S^{\circ}>0$ [22]. Therefore the PEO retention in the RPLC separation is not enthalpy-driven but an entropy-driven process and the PEO retention increases as temperature increases. The peculiar retention behavior of PEO in the RPLC separation can be understood in terms of the hydrophobic interaction of PEO in aqueous mobile phase with the

non-polar stationary phase [27]. The entropy gain due to the release of water molecules from the solvated PEO chain and the stationary phase is believed to be responsible for the entropy-driven sorption process. It could explain the inverted elution order of PEO (high-molecular-mass PEO elutes first) from the positive ΔH^{o} associated with the separation process [27]. Therefore it is not necessary to focus on the size exclusion mechanism to explain the inverted elution order in molecular mass.

In this study, we revisited the RPLC retention behavior of FAEs to elucidate the retention mechanism. For the purpose we investigated the detailed temperature dependence of RPLC retention to obtain the thermodynamic parameters associated with the RPLC retention of FAEs.

2. Experimental

Brij 30 was purchased from Fluka of which the major component is tetraethylene glycol dodecyl ether. The RPLC experiments were carried out with a C_{18} bonded silica column (Luna, Phenomenex, 100 Å, 250×4.6 mm, 5 μm particle size). The mobile phase was a mixture of acetonitrile (Duksan, HPLC grade) and water (deionized and filtered with 0.45 μm pore membrane filter), and the flow-rate was 0.5 ml/min. The FAE samples were dissolved in the mobile phase solvent (10.0 mg/ml) and a 100 μl was injected. The column temperature was controlled by circulating a fluid through a column jacket from a bath/circulator (Neslab, RTE-111). The chromatograms were recorded with a refractive index detector (Shodex, RI-71).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF–MS, Bruker, REFLEX III) was used at an accelerating potential of 20 kV in the reflection mode. The MS instrument was equipped with a nitrogen laser (λ= 337 nm), a pulsed ion extraction, and a reflector. For the low-molecular-mass FAE sample analysis, silica gel (Macherey–Nagel, 500 Å pore, 5 μm particle size) was used as the matrix. The silica gel suspension in methanol (Duksan, HPLC grade) was directly deposited on a MALDI target to form a thin layer, and then the sample solution was placed on the surface of matrix layer and dried at room temperature.

3. Results and discussion

Fig. 1 shows the RPLC chromatograms of Brij 30 obtained at different temperatures. A mixture of acetonitrile–water (70:30, v/v) was used as a mobile phase. We obtained fully resolved chromatograms for both PEO block lengths and alkyl chain lengths in a single isocratic and isothermal RPLC run. The sharp negative peak appearing near t_R=4 min is the injection solvent peak. As clearly observed from the chromatogram taken at 40 °C, the Brij 30 consists of three FAE groups of different alkyl chain length (C_{10}, C_{12}, C_{14}), which elute as well-separated peak envelopes each composed of the elution peaks corresponding to different number of EO units. The middle, major component is the C_{12} species and the next abundant species is the C_{14} species retained longer in the column. C_{10} species exists as a minor component and elute first. All the species were identified by MALDI-TOF–MS.

The overall temperature dependence of the re-

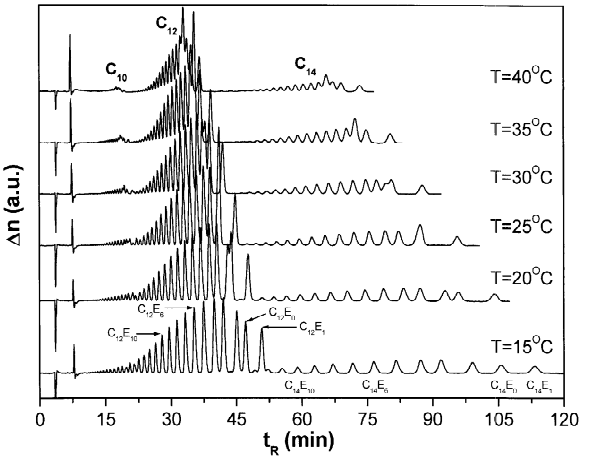

Fig. 1. RPLC chromatograms of Brij 30 taken at various temperatures. The elution peaks were identified from the molar mass measured by MALDI-TOF–MS as shown in Fig. 2 and shown in the chromatogram at 15 °C. It is clear that the FAEs with a constant alkyl chain length elutes in the order of large to small number of EO units. Column: Luna C_{18}, 100 Å pore, 250×4.6 mm, eluent: acetonitrile–water (70:30, v/v) at a flow-rate of 0.5 ml/min.

tention exhibits an interesting behavior. As the temperature changes, average retention time of the peak envelops with a fixed alkyl chain length changes little while the breadth of the envelopes changes markedly. At low temperature, the resolution with respect to the number of EO units is better, but the increased spacing between adjacent peaks yields marked overlap of the elution peaks of the different alkyl groups. As the temperature increases, the resolution according to the number of EO units decreases, but the apparent resolution according to the alkyl chain length is improved. This is analogous to what Jandera et al. observed with the variation of the acetonitrile content [9]. Furthermore, the retention of individual peaks appears not to change monotonically with temperature and it either increases or decreases with the temperature. This is a quite unique behavior rarely found in other polymer systems. This is due to the opposite contribution of alkyl chain and PEO block to the RPLC retention with the aqueous mobile phase as will be discussed in more detail later.

The number of carbon atoms in the alkyl chain (x) and the EO units (y) for each elution peak was determined by MALDI-TOF–MS. For the sake of convenience, each peak of Brij 30 is abbreviated as a C_xE_y. Organic matrices are commonly used for the MALDI-TOF–MS analysis of polymer sample, but organic matrix gives rise to matrix-related background in the low mass region, which makes the analysis of low-molecular-mass samples difficult. We employed silica gel as matrix, which has been successfully applied for the analysis of low-molecular-mass sample [28]. The MALDI-TOF mass spectra of Brij 30 and its fractions are shown in Fig. 2. The measured molecular masses are in good agreement with the calculated molecular mass: $14.03x$ (carbon number in alkyl chain) $+44.05y$ (number of EO units) $+18.02$ (oxygen between PEO and alkyl group, and two hydrogen end groups) $+22.99$ (Na^+ ion). For an example, with $x=12$ and $y=10$ the molar mass of the FAE was found to be 649.5 compared to the calculated mass of 649.9. And from the MALDI-TOF mass spectra, we confirmed that high-molecular-mass EO chain eluted first in this RPLC system, which was already found previously [10,12,13,15,29].

Returning to the temperature dependence of the

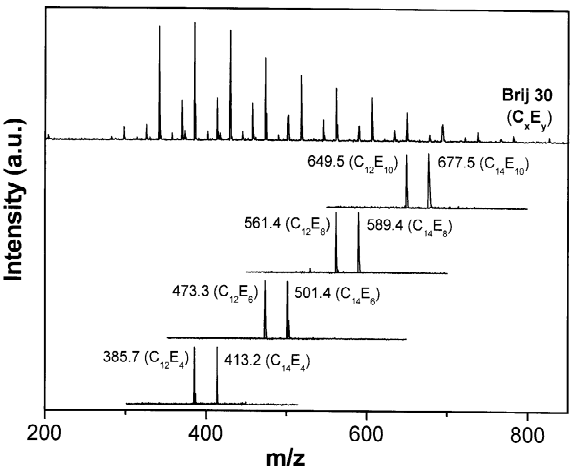

Fig. 2. MALDI-TOF mass spectra of Brij 30 (top) and its fractions (bottom). The molecular mass and the carbon number of alkyl chain (x) and the number of EO units (y) are shown. The MALDI-TOF mass spectra of the fractions were taken for the mixtures of C_{12} and C_{14} species having the same number of EO units. Matrix: bare silica (particle size: 5 μm, pore size: 500 Å), Solvent: methanol.

RPLC retention, the thermodynamic parameters, ΔG^o, ΔH^o, and ΔS^o associated with the sorption process of solutes to the stationary phase can be described by the following relationships.

$$\Delta G^o = \Delta H^o - T\,\Delta S^o = -RT\ln K \quad (1)$$

$$k' \equiv \frac{(V_R - V_o)}{V_o} = K\phi \quad (2)$$

$$\ln k' = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \phi \quad (3)$$

where K is the solute sorption equilibrium constant, k' is the retention factor, ϕ is the volume ratio of stationary phase to mobile phase, V_R and V_o are the retention volume of the solute and the total void volume of the column, respectively. The FAE consists of a PEO block and an alkyl chain. The sorption process of EO units to the non-polar stationary phase showed $\Delta H^o > 0$ and $\Delta S^o > 0$ [9,22,27]. On the other hand, the alkyl chain showed $\Delta H^o < 0$ and $\Delta S^o < 0$ under the same experimental condition [9]. In other words, in the RPLC separation condition, the two moieties of the FAE, the PEO block and the alkyl chain contribute to the retention in the thermody-

namically opposite manner: Sorption of the FAE to the stationary phase is energetically favorable for alkyl chain but unfavorable for PEO block, while it is entropically favorable for PEO block but unfavorable for alkyl chain. Therefore the retention of PEO block increases whereas the retention of alkyl chain decreases with increasing temperature. In results, the retention variation of FAE sample was determined by the relative magnitude of the two contributions. Increasing temperature, the retention of the FAE with a long PEO block increases while the retention of a short PEO block FAE decreases. This explains the general temperature dependence found in Fig. 1 that the average retention of a peak envelope (fixed alkyl chain length) does not change much while the envelope becomes narrower with increasing temperature. However we can find more complex features in the detailed temperature dependence of the RPLC retention.

Fig. 3 displays the van't Hoff plots made from the retention data of C_{12} and C_{14} FAE obtained from the chromatograms shown in Fig. 1. The van't Hoff plots of C_{12} and C_{14} FAE is alike in the overall shape. For the fatty alcohols without EO units attached, $C_{12}E_0$ and $C_{14}E_0$, the van't Hoff plot shows a good linearity and the positive slopes indicate $\Delta H^{o}<0$ as expected from the contribution of alkyl chain. Adding an EO unit to the fatty alcohols increases the retention but further increasing the number of EO units decreases the retention. Also the slope of the plots decreases with addition of EO units indicating the positive enthalpic contribution of EO units. Another interesting feature is that the addition of EO units to the fatty alcohols induces the development of curvature in the van't Hoff plot and it becomes more and more conspicuous as the number of EO units increases. The nonlinear van't Hoff plot indicates that ΔH^{o} (slope of the curve) of the solute sorption changes significantly with temperature. It even changes the sign of ΔH^{o} for the FAE with a large number of EO units at low temperature.

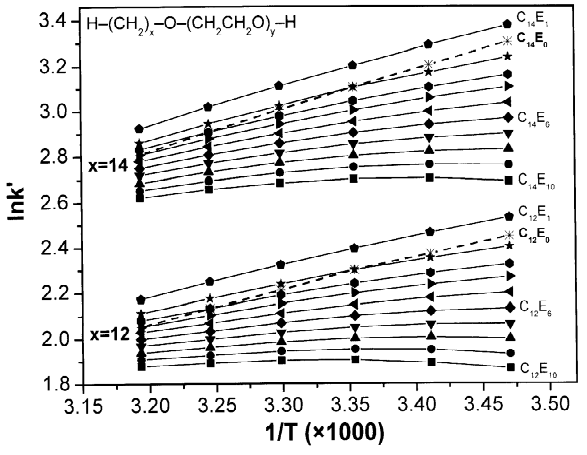

Fig. 3. The van't Hoff plots of C_{12} and C_{14} FAEs constructed from the RPLC chromatograms shown in Fig. 1. The corresponding length of alkyl chain and PEO blocks are also shown in the plot. All the lines are the best fit to Eq. (4). Two dashed lines of $C_{12}E_0$ and $C_{14}E_0$ correspond to aliphatic fatty alcohols, which show a distinctively different retention behavior from FAEs.

For a small temperature range it is common to observe that ΔH^{o}, ΔS^{o} and ϕ are temperature invariant and the plots of $\ln k'$ vs. $1/T$ are linear [30,31]. In this case $\Delta H^{o}$and ΔS^{o}can be obtained from the slope and the intercept of the van't Hoff plot, respectively (Eq. (3)). If the properties of analyte and/or mobile and stationary phase vary with the temperature, the heat capacity of the system may change with temperature leading to variations in $\Delta H^{o}$and $\Delta S^{o}$and nonlinear van't Hoff plots. One can extract the thermodynamic parameters from the tangential slopes and the corresponding intercepts of the nonlinear van't Hoff plot, but the following three-parameter quadratic equation is often used to interpret the nonlinear van't Hoff plot [32].

$$\ln k' = a + \frac{b}{T} + \frac{c}{T^2} + \ln \phi \tag{4}$$

This analysis method of nonlinear van't Hoff plot has been applied for the system involving hydrophobic interaction such as protein folding and stability [33–35], transfer of nonpolar substances from their pure states to water [36], and the hydrophobic interaction chromatography [32]. The three parameters *a*, *b* and *c* can be evaluated by fitting experimental data to Eq. (4). The enthalpy, entropy, and heat capacity changes can be calculated with the parameters by using the following equations.

$$\Delta H^{o} = -R\frac{\mathrm{d}\ln k'}{\mathrm{d}(1/T)} = -R(b + \frac{2c}{T}) \tag{5}$$

$$\Delta S^{*} = \Delta S^{o} + \ln \phi = R(a - \frac{c}{T^2}) + \ln \phi \tag{6}$$

$$\Delta Cp^{o} = 2R\frac{c}{T^{2}} \quad (7)$$

The nonlinear van't Hoff plots in Fig. 3 were fitted to Eq. (4) to extract the thermodynamic parameters. The solid lines in Fig. 3 are the best-fit results. Using the thermodynamic parameters obtained from Eqs. (5)–(7), we plotted the temperature dependences of ΔCp^{o}, ΔH^{o} and ΔS^{*} for the C_{12}FAE in Fig. 4a,b and c, respectively. All the thermodynamic parameters appear to follow the relationships, Eqs. (5)–(7) very well. Fig. 4a shows that ΔCp^{o} of C_{12} FAE is not independent of temperature, which results in the variation of ΔH^{o} and ΔS^{*} with temperature. As can be seen in Fig. 4b and c, both ΔH^{o} and ΔS^{*} increase as the temperature decreases and even they change the sign. At low temperature and a large number of EO units, both ΔH^{o} and ΔS^{*} are positive, which results from the dominating hydrophobic interaction effect of relatively long PEO block length. And the magnitude of ΔH^{o} is relatively small and ΔS^{*} dominantly controls the separation process. On the other hand, for the FAEs with a small number of EO units, ΔH^{o} and ΔS^{*} become both negative since the contribution of alkyl chain becomes larger. In addition, the magnitude of $\Delta H^{o}$is large indicating that ΔH^{o} becomes the major controlling factor on the retention of the FAE sample.

We have also examined the dependences of thermodynamic parameters on the number of EO units as well as carbon number in alkyl group. Fig. 5 displays the molecular mass dependences of ΔCp^{o}, ΔH^{o} and ΔS^{*} at 25 °C. The ΔCp^{o} decreases precipitously by adding first two EO units to the alkyl chain and then gradually decreases with further increase of the number of EO units. In parallel, the dependence of ΔH^{o} and ΔS^{*} on the number of EO units shows a break at near two or three EO units. Otherwise both ΔH^{o} and ΔS^{*} show a good linear relationship with the number of EO units. Fig. 5b shows that about 2.4 kJ/mol difference in ΔH^{o} persists between C_{12} and C_{14} FAEs independent of the number of EO units. The magnitude of ΔH^{o} per methylene unit, -1.2 kJ/mol is comparable to ΔH^{o} per EO unit, which can be deduced from the slope of the plots, $+1.17$ kJ/mol. On the other hand, as shown in Fig. 5c, the difference in ΔS^{*} is only about -1.4 J K^{-1} mol^{-1} between C_{12} and C_{14} FAEs much less in magnitude than $+3.5$ J K^{-1} mol^{-1} per EO unit.

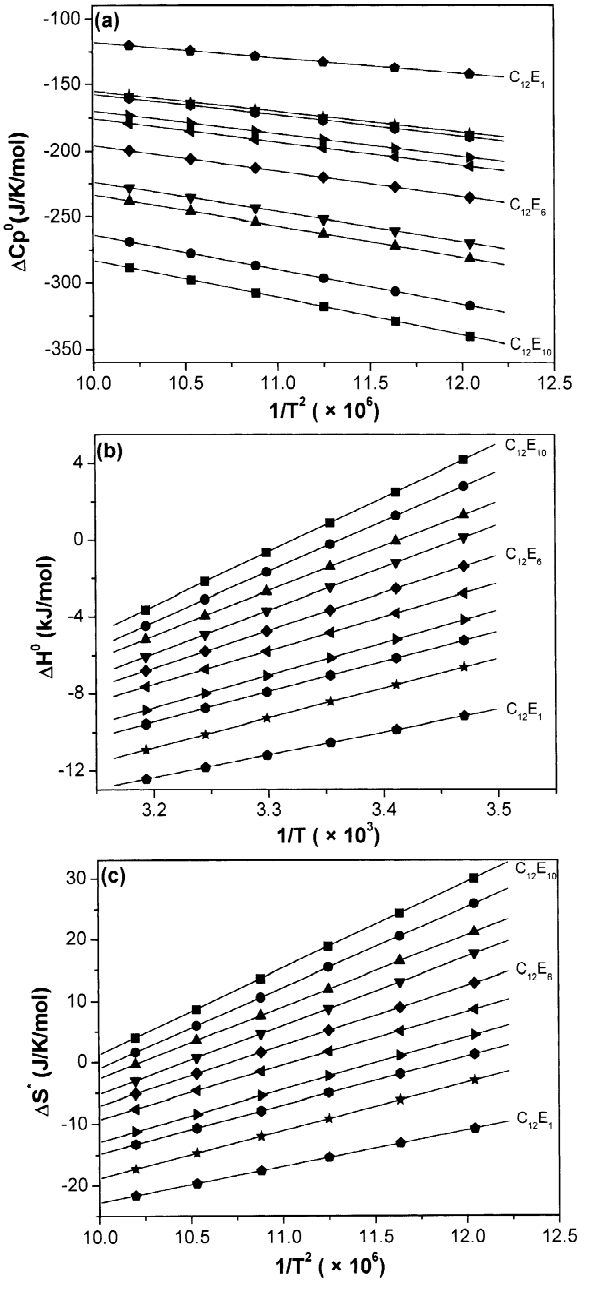

Fig. 4. Temperature dependence of ΔCp^{o}(a), ΔH^{o} (b), and ΔS^{*} (c) associated with the retention of C_{12} FAEs. The thermodynamic parameters are obtained from Eqs. (5)–(7). All the plots for the three parameters show good linearity according to Eqs. (5)–(7).

Therefore at this separation condition near room temperature, increase of the carbon number in the alkyl chain should increase the retention significantly

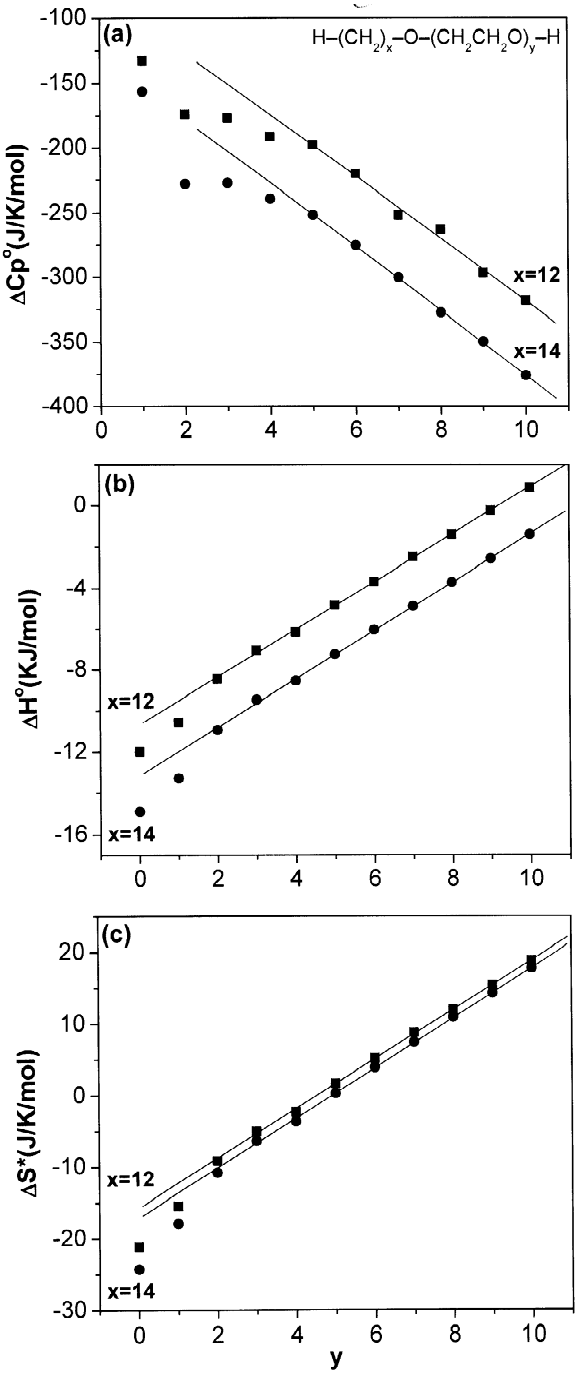

Fig. 5. Dependence of ΔCp^{o}(a), ΔH^{o} (b), and ΔS^{*} (c) on the number of EO units for C_{12} and C_{14} FAEs at 25 °C. Solid lines are drawn for visual aid.

due to the strong enthalpic contribution of the methylene unit. On the other hand, enthalpic and entropic contribution of an EO unit to the retention more or less compensate each other and results fine splitting of the peaks as can be seen in Fig. 1. Also these thermodynamic parameter values indicate that the inverted elution order of the FAE with respect to the number of EO units is caused by the positive ΔH^{o} per EO unit since the positive ΔS^{*} per EO unit should have increased the retention of the FAE with the number of EO units. The inverted elution order reflects the dominance of the enthalpic effect of EO unit and the effect is more pronounced at low temperature as easily inferred from Eq. (3). In any event, it is clear that the inverted elution order has nothing to do with the size-exclusion effect.

In summary, we obtained fully resolved chromatograms of Brij 30 in a single isocratic and isothermal RPLC run. The general feature of the temperature dependence of the FAE retention in the RPLC column can be interpreted considering the opposite thermodynamic contributions of the PEO block and the alkyl chain. However, the temperature dependence of the retention exhibits nonlinear van't Hoff plots indicating that the thermodynamic parameters associated with the RPLC separation of FAE deviates from the simple summation of the contributions of the homo-PEO and aliphatic hydrocarbon, both of which show linear van't Hoff plots [22,27]. The nonlinear van't Hoff plots are well explained by considering the variation of the thermodynamic parameters with temperature.

In addition, the thermodynamic analysis of the RPLC retention of FAEs shows unequivocally that the inverted elution order of FAEs is caused by the positive ΔH^{o} per EO unit. The unusual retention behavior of FAEs is due to the concerted contribution of the hydrophobic interaction for PEO block ($\Delta H^{o}>0$, $\Delta S^{o}>0$) and the enthalpic interaction of alkyl chain ($\Delta H^{o}<0$, $\Delta S^{o}<0$) with the reversed-phase stationary phase in the aqueous–organic mobile phase medium.

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