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# Retention of 3,5-dinitrobenzoyl derivatives of linear alcohol polyethoxylates in reversed-phase liquid chromatographic system with acetonitrile–water mobile phase

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**Abstract**

The influence of mobile phase composition (acetonitrile–water ratio) on the separation of derivatised linear alkyl polyethoxylates (LAEs) is evaluated using thermodynamic quantities (Gibbs energy, enthalpy and entropy). In comparison to homologue series of alcohols oligomers of LAEs show irregular chromatographic behaviour that is demonstrated in irregular changes of thermodynamic quantities. It might be explained considering an influence of some of the following processes or their combinations on the retention of LAEs. These processes are solvation of oxyethylene chains in mobile phase, their interaction with silanols on silica surface of stationary phase and possibly their conformation changes. The composition of a mobile phase affects the mentioned processes and that is why the retention of LAEs is strongly (for the reversed-phase system unusually) sensitive to this composition in the studied range (volume fraction of acetonitrile $\varphi = 0.90$, 0.96 and 0.98). The experimental data also support the idea of the active role of stationary phase in the reversed-phase system.


*Keywords:* Retention behaviour; Mobile phase composition; Polyethoxylates; Surfactants

## 1. Introduction

The analysis of linear alkyl polyethoxylates (LAEs) is an important task regarding to their widely spread use as non-ionic surfactants. One of the effective methods in this field is high performance liquid chromatography (HPLC) [1–5]. This technique was used for the so-called separation of homologues (separation according to alkyl chain length) as well as oligomers (separation according to the number of oxyethylene units) of LAEs. These compounds contain a linear primary alkyl chain with mainly 11–17 methylene groups ($m$) and an oxyethylene chain typically with an average ethoxylation degree 5–10 ($n$ – number of oxyethylene units).

$CH_3(CH_2)_m–(O–CH_2–CH_2)_n–OH$

For the sensitive determination of LAEs using UV or fluorescent detection the compounds have to be derivatised. Earlier, we studied the separation of homologues of LAEs derivatised by 1-naphthyl isocyanate [6] and 3,5-dinitrobenzoyl chloride (3,5-DNBCl) [7] in reversed-phase HPLC (RP-HPLC) system. The sufficient homologue by homologue separations with the suppression of the oxyethylene chain influence were reached. The effect of the different parameters (nature and content of an organic solvent, salt concentration, temperature) on separation was described. We recognised the strong influence of the mobile phase composition in the certain range on the LAE retention. Two chromatographic features of the studied system were essential for the required separation, the change of the elution order of oligomers (ethoxymers) in the used interval of mobile phase composition and the increase of the retention of higher LAE oligomers with increasing content of an organic solvent in the binary (water–organic solvent) mobile phase.

The irregular chromatographic behaviour of ethoxylated compounds some authors explained by the influence of equilibrium of conformers of oxyethylene chains. Melander and co-authors described the chromatographic behaviour of ethoxylated compounds (not LAEs) in RP-HPLC using

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two stable conformers equilibrium [8]. They stated that this equilibrium is affected by eluent composition, temperature and the number of oxyethylene units in the molecule. For RP-HPLC system Okada interpreted the retention behaviour of ethylenglycol and dialkyl ethylenglycol oligomers assuming equilibrium of two stable conformers (more polar gauche and less polar trans) [9]. He employed the retention model in combination with molecular mechanics calculation. He also stated some limitation of the used method (e.g. impossibility to calculate changes of entropy). Kamiusuki et al. [10] also explained anomalous retention behaviour of lauryl alcohol ethoxylates on polyfluoroalkylsilane coated silica gel column by mentioned conformational change. Differences between separation of the studied compounds on endcapped and non-endcapped columns they ascribed to the interaction of the oxyethylene chain with residual silanols. Nahum and Horváth described irregular retention of crown ethers (compounds with some analogies to polyethylenglycols) in RP-HPLC [11]. They interpreted the phenomenon by the interaction between analytes and the accessible silanol groups at the surface of alkyl bonded silica stationary phase. On the contrary Jandera et al. [12] explained the retention of ethoxylated alcohols by solvation effects of the oxyethylene groups.

Co-elution of the oligomers of ethoxylated alcohols (separation only according to their alkyl chain length) was also explained by elution at the critical point of adsorption, limiting case between size exclusion and adsorption modes when the entropic and enthalpic contributions compensate each other [13].

In the presented work, the temperature dependencies of retention factors are used for the determination of thermodynamic characteristics of the separation process. The influence of acetonitrile–water mobile phase composition on the chromatographic behaviour of LAEs derivatised by 3,5-DNBCl in RP-HPLC system is evaluated. The mechanism of the separation of alcohols (homologues) and ethoxylates (oligomers, ethoxymers) is compared using the changes of enthalpy, entropy and Gibbs energy.

## 2. Experimental

### 2.1. Chromatographic experiments

The chromatographic work was performed using a Spectra-Physics liquid chromatograph (pump SP 8700, UV–Vis absorption detector SP 8440, all Spectra-Physics, San Jose, CA, USA) equipped with a manual 7125 injector (10 μl loop) (Rheodyne, Cotati, CA, USA) and Chromatography Station for Windows CSW version 1.0 (DataApex, Prague, Czech Republic). The chromatograms of derivatised LAEs were recorded at 233 nm.

The separations were carried out by means of a 250 mm × 4 mm i.d. LiChrospher 100 RP-18 endcapped 5 μm LiChroCart column (E. Merck, Darmstadt, Germany). The temperature of the column was controlled with a precision of ±0.1 °C using a glass water jacket and a laboratory water thermostat equipped with a freon cooler.

The mobile phases were prepared by mixing accurately weighed components (HPLC-grade acetonitrile (E. Merck, Darmstadt, Germany) and redistilled water). The flow-rate was 1.5 ml/min.

The retention times were measured at least in two injections. The injection was repeated more times when the difference of experimental values from an average was higher than 0.35%. Hold-up times were determined by at least three injections of methanol (0.5 μl) after each change of experimental conditions at 200 nm. After the subtraction of time corresponding to extra column volumes the mean values of retention as well as hold-up times were used for calculation of retention factors.

Linear primary $C_{11}$—$C_{16}$ and $C_{18}$ alcohols ($C_{11}$OH—$C_{16}$OH, $C_{18}$OH) (all Aldrich, Milwaukee, WI, USA), diethylenglycol ($C_{12}E_2$), tetraethylenglycol ($C_{12}E_4$), hexaethylenglycol ($C_{12}E_6$), heptaethylenglycol ($C_{12}E_7$), oktaethylenglycol ($C_{12}E_8$), nonaethylenglycol ($C_{12}E_9$) monododecyl ethers (all Fluka, Buchs, Switzerland) and Marlipal 28/100 (mixture of even linear primary fatty alcohol ($C_{12}$–$C_{18}$) ethoxylates with an average number of 10.2 oxyethylene units; gift Prof. A. Marcomini, University of Venice, Venice, Italy) were used as solutes. The derivatisation reaction was performed, as was described earlier [7]. The working solutions were prepared in acetonitrile and their concentration was ≈0.1 mg of underivatised compound/ml (≈5 mg of underivatised sample/ml in the case of Marlipal 28/100). Injection volume was 10 μl.

### 2.2. Thermodynamic calculation

For the study of chromatographic mechanism, the evaluation of thermodynamic characteristics can be used. If the phase ratio $\Phi = V_s/V_m$ is known ($V_s$ is the volume of stationary phase, $V_m$ is the volume of mobile phase) Van't Hoff relationship (1) would allow the calculation of the change of enthalpy ($\Delta H^\circ$) as well as entropy ($\Delta S^\circ$).

$$\ln k = -\left(\frac{\Delta H^\circ}{RT}\right) + \left(\frac{\Delta S^\circ}{R}\right) + \ln \Phi, \quad (1)$$

what corresponds to

$$\ln k = A + \frac{B}{T}, \quad (2)$$

where $k$ is retention factor, $T$ is absolute temperature, $R$ is universal gas constant, $A$ and $B$ are the coefficients of experimentally determined relationship. As the phase ratio in our experiments was unknown we calculated following differences.

$$\Delta(\Delta S^\circ) = (A_x - A_{C_{12}OH})R \quad (3)$$

$$\Delta(\Delta G^\circ) = -RT \ln\left(\frac{k_x}{k_{C_{12}OH}}\right), \quad (4)$$

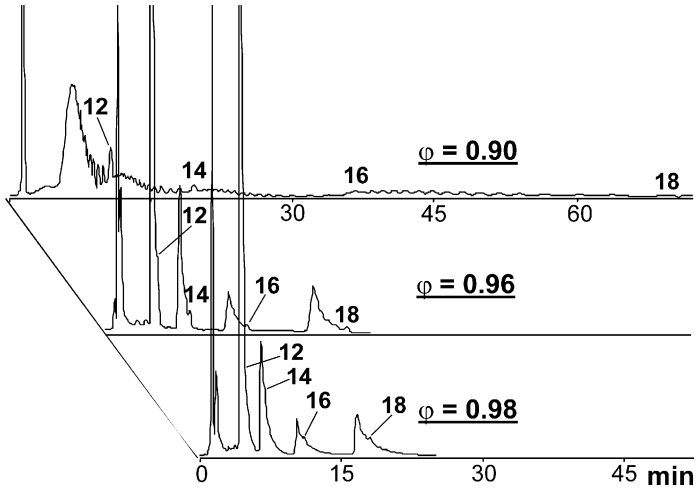


Fig. 1. Chromatograms of Marlipal 28/100 obtained with mobile phases differing in acetonitrile content. Stationary phase: LiChrospher 100 RP-18, endcapped, 5 μm; column: LiChroCart 250 mm × 4 mm i.d.; mobile phase: acetonitrile–water ($\varphi$ is the volume fraction of acetonitrile); flow-rate 1.5 ml/min; temperature 20 °C; detection: UV at 233 nm (derivatised compounds); injection volume 10 μl; working solutions in acetonitrile (≈5 mg of underivatised sample/ml). Numbers in chromatograms show elution of corresponding derivatised alcohols (12, $C_{12}OH$; 14, $C_{14}OH$; 16, $C_{16}OH$; 18, $C_{18}OH$).

where $k_x$ and $k_{C_{12}OH}$ are the retention factors of solute and $C_{12}OH$ respectively. Subscript 'x' means some of the studied compounds. $C_{12}OH$ is used for comparison as one of the alcohol homologues and as the "zeroth" member of oligomeric series of LAEs with dodecyl group.

## 3. Results and discussion

Chromatograms of Marlipal 28/100 obtained with different mobile phases (see Fig. 1) show that the change of acetonitrile content leads to the important differences in relative retention of LAEs. While for $\varphi = 0.90$ alcohols are eluted in the end of corresponding group of oligomers (the retention is decreasing with longer oxyethylene chain) and substantial overlap of homologues occurs (e.g. $C_{12}OH$ as well as some $C_{12}$ ethoxylates are eluted between $C_{14}$ ethoxylates), for $\varphi = 0.96$ the homologues are well separated. Corresponding alcohols still prove elution in the end of bunches of oligomers. Other increase of acetonitrile content ($\varphi = 0.98$) makes homologue separation worse, bunches of oligomers are more tailing (compare chromatograms for $\varphi = 0.96$ and $\varphi = 0.98$, Fig. 1) and higher ethoxymers are more strongly retained than alcohol with the same alkyl chain. This behaviour was mentioned in our previous papers [6,7] and it has to be kept in mind if separation of LAEs according their alkyl chain has to be achieved.

The evaluation of chromatographic behaviour of derivatised LAEs was performed using experimentally determined relationships (2) between natural logarithm of retention factor and reciprocal value of absolute temperature. These dependencies were measured for series of standards (alcohols $C_{11–16}OH$ and $C_{18}OH$, ethoxymers $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_{6–9}$) in three different mobile phases and in the temperature range from 5.0 to 72.5 °C for 10 values (step 7.5 °C). The statistical evaluation showed that the experimental data are well described by linear functions (the worst correlation coefficient was 0.9956). The thermodynamic quantities were then calculated but due to unknown phase ratio of the utilised column we have to relate $\Delta G^\circ$ and $\Delta S^\circ$ to one compound ($C_{12}OH$) (see experimental section).

The Figs. 2 and 3 show courses of the $\Delta(\Delta G^\circ)$ dependencies on the number of structural units $m$ (methylene groups for alcohols) and $n$ (oxyethylene groups for ethoxymers), respectively.

As regards the dependencies for alcohols only one of them (for $\varphi = 0.96$, $\varphi$ is the volume fraction of acetoni-

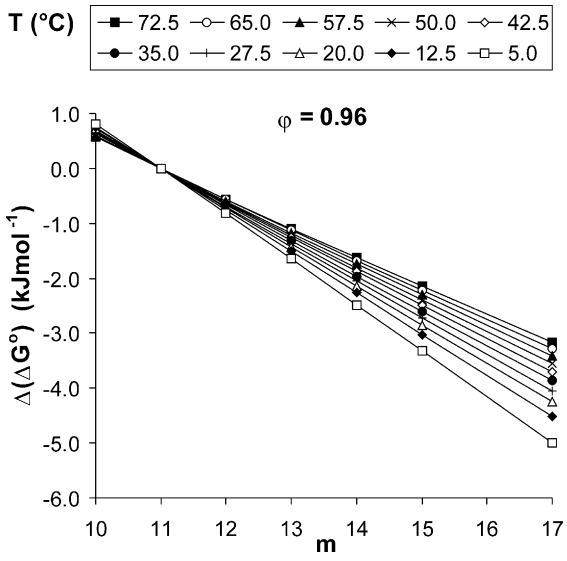


Fig. 2. The dependence of $\Delta(\Delta G^\circ)$ on the number of methylene groups ($m$) of studied alcohols ($C_{11–16}OH$ and $C_{18}OH$). Working solutions in acetonitrile (≈0.1 mg of underivatised compound/ml); temperature from 5 to 72.5 °C; other conditions, see Fig. 1.

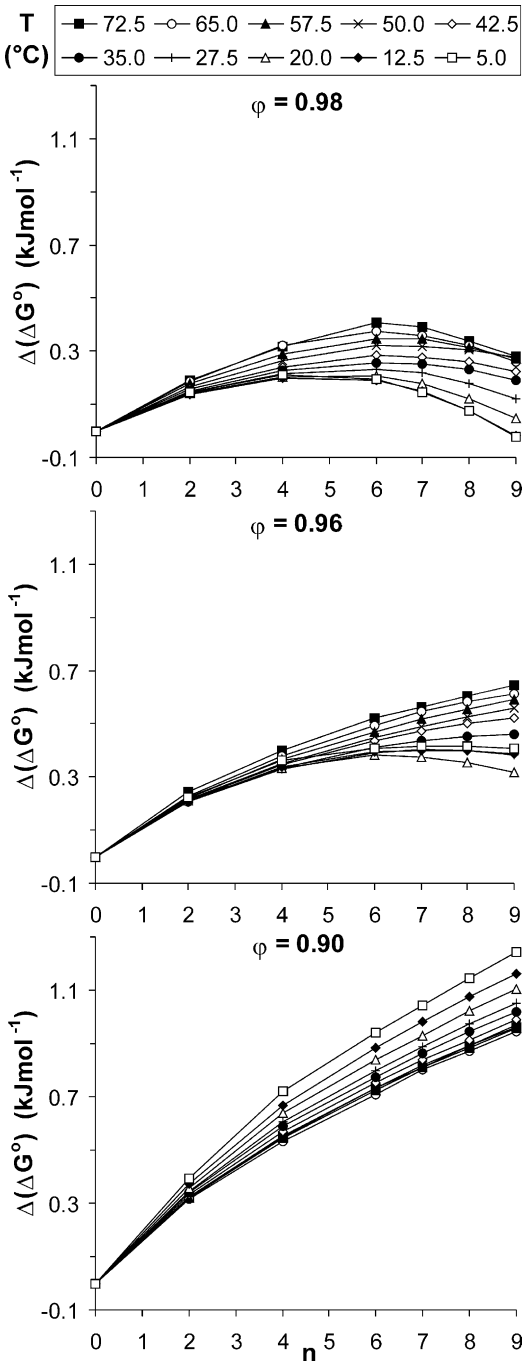


Fig. 3. The dependence of $\Delta(\Delta G^\circ)$ on the number of oxyethylene groups ($n$) of studied ethoxymers ($C_{12}OH$, $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_{6–9}$). For conditions, see Fig. 2.

trile) is shown (Fig. 2) because they are analogous in the different mobile phase compositions. Their shapes are typical for well-known chromatographic behaviour of the members of homologue series. The retention of compounds increases with longer alkyl chain (more negative $\Delta(\Delta G^\circ)$ values, higher absolute values of $\Delta(\Delta G^\circ)$), decreases with higher temperature as well as with higher content of acetonitrile in the mobile phase (less negative $\Delta(\Delta G^\circ)$ values, lower absolute values of $\Delta(\Delta G^\circ)$). For the particular experimental conditions (column, mobile phase, temperature), the change of $\Delta(\Delta G^\circ)$ per one methylene group is not very different in homologue series of studied alcohols (the biggest difference is 74 J/mol for maximal (−555 J/mol) and minimal (−481 J/mol) $\Delta(\Delta G^\circ)$ per one methylene group at 72.5 °C and $\varphi = 0.98$). With the increase of water content this difference lowers, e.g. for the same temperature and $\varphi = 0.90$ it is 39 J/mol for maximal (−644 J/mol) and minimal (−605 J/mol) $\Delta(\Delta G^\circ)$ per one methylene group. Hence the observed selectivity of separation of consecutive homologues is almost constant for given conditions, increases with higher content of water in mobile phase and decreases with higher temperature.

Wholly different situation occurs in the case of ethoxymers (Fig. 3). The shape of plots of $\Delta(\Delta G^\circ)$ versus number of oxyethylene units is dependent on the composition of mobile phase. It is very sensitive to the content of water in the mobile phase. At $\varphi = 0.90$ $\Delta(\Delta G^\circ)$ increases with n for all standards of LAEs, at $\varphi = 0.98$ $\Delta(\Delta G^\circ)$ firstly increases but for higher n starts to fall. That is evidence of the change of the separation process. The partial change of elution order is apparent from chromatograms in Fig. 1 too. The processes mentioned in the introduction might be the cause of the irregular behaviour of LAEs. The shift in conformer equilibrium [8–10] to less polar conformer(s) of oxyethylene chain that shows higher retention in reversed-phase system, the rise of interaction between oxyethylene chain and residual silanol groups [10] as well as the changes in solvatation of oxyethylene chain [12] with the lowering of water content, each of these processes can lead to the described retention behaviour of derivatised LAEs.

In Fig. 3, we can also see effect of temperature on relative retention. At $\varphi = 0.98$ the predominant trend is the increase of $\Delta(\Delta G^\circ)$ values with higher temperature. In other words, the retention of ethoxymers decreases more with growing temperature than the retention of $C_{12}OH$. However it can be also seen that lower temperatures (5 and 12.5 °C) allow a slightly later elution of $C_{12}E_9$ in comparison to $C_{12}OH$. The situation is opposite at $\varphi = 0.90$. The predominant trend is decrease of $\Delta(\Delta G^\circ)$ values with higher temperature. The discussed change in the mentioned trend is important difference compared to alcohols for which $\Delta(\Delta G^\circ)$ (relative retention) drops with higher temperature at all φ values. This allows to use effectively temperature in the optimisation of homologue by homologue separation (to increase the homologue separation and in the same time to keep zones of ethoxymers with the same alkyl relatively narrow) [7].

Fig. 3 shows lower deformation of dependencies of standards for more polar mobile phase. At $\varphi = 0.90$ their courses are monotonic for all temperature values what manifests lesser influence of process(es) inducing irregular retention applying mobile phases with higher content of water. Mechanism of retention of ethoxymers gradually changes as function of temperature, water content in mobile phase and number of oxyethylene units.

For the used experimental conditions, it is not possible to reach the co-elution of all $C_{12}$ oligomers. In such case, the condition $\Delta(\Delta G^{\circ}) = 0$ for them has to be valid. The increase of acetonitrile content in the mobile phase can lead to higher retention of lower ethoxymers to obtain their co-elution with $C_{12}OH$ but the experiments with Marlipal 28/100 showed that already at 72.5 °C ($\varphi = 0.98$) some higher ethoxymers are eluted later than $C_{12}OH$. Higher content of acetonitrile in the mobile phase (as well lower temperature) can only increase their retention in comparison to $C_{12}OH$. That is why we cannot expect the co-elution of LAEs with the same alkyl chain exactly in the same retention time.

The Figs. 4 and 5 (the changes of enthalpy and entropy) offer more detail evaluation of LAE retention. For alcohols, the course of dependencies is monotonic (Fig. 4). Higher number of methylene groups means more negative enthalpy (more exothermic process). The retention of molecules with longer non-polar alkyl chain is in term of enthalpy more convenient. The change of enthalpy for alcohols is more negative with lower content of water in mobile phase. The entropy also decreases with longer alkyl chain. This is evidence of the active role of stationary phase in chromatographic process [14–16]. The transfer of solute with longer alkyl chain to the stationary phase leads to the relatively more ordered system. Alkyl chains in the alcohol molecules penetrate into stationary phase and longer alkyls more restrict the conformation freedom of stationary phase chains.

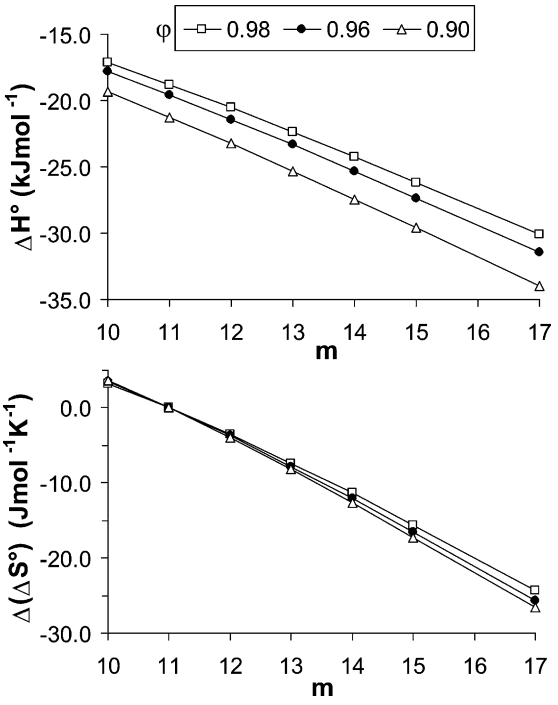


Fig. 4. The enthalpic and entropic changes vs. the number of methylene groups ($m$) of studied alcohols ($C_{11–16}OH$ and $C_{18}OH$). For conditions, see Fig. 2.

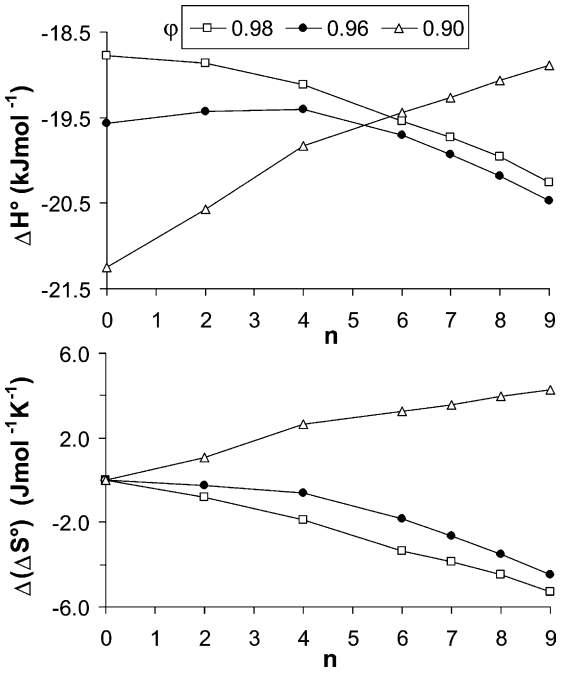


Fig. 5. The enthalpic and entropic changes vs. the number of oxyethylene groups ($n$) of studied ethoxymers ($C_{12}OH$, $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_{6–9}$). For conditions, see Fig. 2.

With lengthening of alkyl chain in alcohol molecule the contribution of enthalpy (supports retention) functions against the contribution of entropy (impacts against retention) but in all studied cases the change of Gibbs energy is more negative for higher homologues (they are more retained).

More complicated situation was observed in the case of ethoxymers (see Fig. 5). The course of dependencies alters dramatically with the relatively small change of water content. As regards enthalpy the transfer of solute to the stationary phase is less favourite in comparison to $C_{12}OH$ for water richer mobile phases. This fact can be recognise for lower ethoxymers already at $\varphi = 0.96$, for all measured ethoxymers at $\varphi = 0.90$. As we move to less polar mobile phase the interactions of oxyethylene groups start to contribute to the increase of retention of analytes.

With the longer oxyethylene chain the ethoxymer retention becomes less convenient in term of entropy in the mobile phases containing higher fraction of acetonitrile ($\varphi = 0.98$ or 0.96) (see Fig. 5). In comparison to $C_{12}OH$, the system is more ordered when ethoxymers are retained in the stationary phase. In acetonitrile rich mobile phases, we expect lesser ordering of solvent molecules around analyte molecule because hydrogen bond interactions of solvents are rather limited in these mobile phases. The ordering is important for solvophobic (or more concretely hydrophobic) effect that is thus more significant in mobile phases with higher content of water [17]. We presuppose mainly influence of restriction of conformation freedom of stationary phase chains similarly as for retention of alcohols. Discussed results again support the

idea of active role of stationary phase in the reversed-phase system. On the contrary at $\varphi = 0.90$ the retention of higher ethoxymers is entropically more convenient. It might be result of lesser penetration of higher ethoxymers into the stationary phase as well as higher ordering of solvent molecules in solvation shell of oxyethylene chain in the mobile phase. It is evident for $\varphi = 0.90$ and 0.98 that in the process of LAE retention the contribution of enthalpy functions against the contribution of entropy (Fig. 5). At $\varphi = 0.90$ with lengthening of oxyethylene chain the retention decreases, the effect of enthalpy predominates the effect of entropy. In the mobile phase with low-content of water ($\varphi = 0.98$), ethoxymer standards (except $C_{12}E_9$ at 5 and 12 °C) show lower retention than $C_{12}OH$ (higher retention in comparison to $C_{12}OH$ was observed for higher ethoxymers from Marlipal 28/100 especially at lower temperatures). For these standards, the contribution of enthalpy (supports retention, at $\varphi = 0.98$) is prevailed by contribution of entropy (impacts against retention, at $\varphi = 0.98$) but for higher ethoxymers the contribution of enthalpy overbalances.

## 4. Conclusion

In comparison to alcohols (homologues), LAEs (oligomers) exhibit very different chromatographic behaviour. The changes of thermodynamic characteristics are in agreement with previously described separation of LAE derivatives and observed chromatographic behaviour in the reversed-phase system (the change of elution order and the increase of retention with the decrease of mobile phase polarity) [6,7]. Such behaviour might be explained by the influence of solvation of oxyethylene chains in mobile phase, their interaction with silanols on silica surface of stationary phase and possibly by their conformation changes. These processes are affected by mobile phase composition (ratio of acetonitrile–water). Their relative importance cannot be estimated from obtained data. It might be that the processes are not independent, e.g. the change of solvation can lead to the change of conformation and different conformers can prove different distribution between stationary and mobile phase. Besides that the active role of stationary phase (decrease of conformation freedom of alkyl chains bonded to silica, i.e. decrease of entropy due to the penetration of analytes to the stationary phase) influences the retention of studied compounds.

On the basis of obtained data we can conclude that for studied LAE derivatives it is not possible to find such experimental conditions in our chromatographic system leading to the co-elution of all ethoxymers with the same alkyl chain in one retention time. Nevertheless, after careful optimisation of mobile phase (acetonitrile–water ratio) the elution of oligomers with the same alkyl chain in the narrow zone is possible.

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## References

[1] W. Miszkiewicz, J. Szymanowski, Crit. Rev. Anal. Chem. 25 (1996) 203–246.
[2] M. Zanette, A. Marcomini, E. Marchiori, R. Samperi, J. Chromatogr. A 756 (1996) 159–174.
[3] K. Rissler, J. Chromatogr. A 742 (1996) 1–54.
[4] T. Cserhati, E. Forgacs, J. Chromatogr. A 774 (1997) 265–279.
[5] C. Vogt, K. Heinig, Fresenius J. Anal. Chem. 363 (1999) 612–618.
[6] K. Lemr, M. Zanette, A. Marcomini, J. Chromatogr. A 686 (1994) 219–224.
[7] K. Lemr, J. Chromatogr. A 732 (1996) 299–305.
[8] W.R. Melander, A. Nahum, Cs. Horváth, J. Chromatogr. 185 (1979) 129–152.
[9] T. Okada, Anal. Chim. Acta 281 (1993) 85–93.
[10] T. Kamiusuki, T. Monde, K. Omae, K. Morioka, T. Konakahara, Chromatografia 51 (2000) 390–396.
[11] A. Nahum, Cs. Horváth, J. Chromatogr. 203 (1981) 53–63.
[12] P. Jandera, M. Holčapek, G. Theodoridis, J. Chromatogr. A 813 (1998) 299–311.
[13] B. Trathnigg, A. Gorbunov, J. Chromatogr. A 910 (2001) 207–216.
[14] J.G. Dorsey, K.A. Dill, Chem. Rev. 89 (1989) 331–346.
[15] M.R. Böhmer, L.K. Koopal, R. Tijssen, J. Phys. Chem. 95 (1991) 6285–6297.
[16] R. Tijssen, P.J. Schoenmakers, M.R. Böhmer, L.K. Koopal, H.A.H. Billiet, J. Chromatogr. A 656 (1993) 135–196.
[17] Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129–156.