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Reversed-phase high-performance liquid chromatographic separation of 1-naphthyl isocyanate derivatives of linear alcohol polyethoxylates

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Abstract

The optimization procedure for the reversed-phase HPLC separation of 1-naphthyl isocyanate derivatives of linear alcohol polyethoxylates (LAEs) is reported. Using a C_{18}-bonded silica stationary phase and acetonitrile–water mixtures as mobile phase, different trends in the chromatographic separation of selected 1-tetradecanol polyethyleneglycol ether ethoxymers were observed. In the investigated range of acetonitrile–mobile phase volume ratio (φ), the elution order of the ethoxymers was inverted by increasing the organic solvent content of the mobile phase, and the mobile phase composition was found which provides the co-elution of the tested compounds. On the basis of the trends of capacity factor logarithm ($\log k'$) versus the number of ethoxy units (n) at different φ, an increased retention by increasing φ was also predicted for higher ethoxymers. The separation under the same chromatographic conditions of a C_{12}–C_{18} LAE mixture with an average number of 10 ethoxy units, confirmed the strong variability of LAE chromatographic behaviour in the investigated φ range. The isoeluting conditions found for the C_{14} LAE ethoxymers were also applied successfully to the homologue-by-homologue separation of the C_{12}–C_{18} LAE mixture.

1. Introduction

The non-ionic surfactants of the linear alcohol polyethoxylate (LAE) type are widely used in both domestic and industrial detergent formulations. During the early 1990s they accounted for more than 50% of the total annual production of non-ionics ($500 \cdot 10^6$ kg in 1991 [1]). The hydrophobic part of LAEs is represented by linear, primary alkyl chains with 10 to 18 carbon atoms including both even and odd homologues for oxo-LAEs and only even homologues for oil-derived LAEs. Industrially produced oxoalcohol polyethoxylates contain also slightly branched primary alcohols in variable proportion (10–50%), depending on the synthesis conditions. Each homologue itself consists of a mixture of oligomers differing by the number of monomeric units in the polyethoxy chain. LAEs used for detergency purposes typically present an average ethoxylation grade of 5–10.

The determination of LAEs by reversed-phase high-performance liquid chromatography (RP-

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HPLC) [2–12] in biodegradation test liquors, as well as environmental samples, requires adequate derivatization. The proposed derivatizing agents were so far the UV-absorbing phenyl isocyanate [2–7] and 3,5-dinitrobenzoyl chloride [8], and the fluorescent 1-anthroylnitrile [10], 1-naphthyl isocyanate [11] and the naphthoyl chlorides [12]. Derivatization by fluorescent agents is preferable for environmental analysis particularly because of the increased selectivity. Modification of LAE molecule through derivatization can change remarkably the partitioning between mobile and stationary phases of each ethoxymer with the same alkyl chain. For determining LAEs in environmental samples, where the concentration is typically very low and matrix interferences are present, the achievement of high sensitivity and the easy peak identification in the resulting RP-HPLC chromatogram is a quite important goal which can be attained by the so called "homologue-by-homologue" separation, i.e. by co-eluting all the oligomers of each homologue. In this work we focus on the optimization procedure for the homologue-by-homologue separation of the 1-naphthyl isocyanate (NIC) derivatives of LAEs.

Once defined the chromatographic retention in terms of capacity factor k' (Eq. 1):

$$k' = (V_R - V_m)/V_m \quad (1)$$

where V_R is the retention volume of a given compound and V_m is the dead volume of the column, the relationship between log k' and the mobile phase composition can be expressed in RP-HPLC by linear or quadratic relationships [13,14] such as:

$$\log k' = a - m\varphi \quad (2)$$

$$\log k' = a - m\varphi + d\varphi^2 \quad (3)$$

where φ is the organic solvent fraction (v/v) in the mobile phase and a, m and d are constants. For homologue or oligomer series, relationships were reported [15,16] between log k' and a number of repeating structural units, n, of the type:

$$\log k' = \log \beta + n \log \alpha \quad (4)$$

$$\log k' = \log \beta + n \log \alpha + n^2 \log \gamma \quad (5)$$

The knowledge of the constants in the Eqs. 2–5 allows the prediction of the capacity factors of different members of homologue or oligomer series under different compositions of mobile phase. We determined experimentally the constants of Eq. 2 measuring the capacity factors of the NIC derivatives of the *n*-tetradecanol and a series of individual ethoxymers of the C_{14} LAE homologue at different φ. Eq. 2 was then used for the calculation of the mobile phase composition allowing the optimal ethoxymer co-elution. The same mobile phase was finally applied to the homologue-by-homologue separation of a C_{12}–C_{18} LAE mixture after derivatization with NIC.

2. Experimental

The chromatographic work was carried out by a 1050 series liquid chromatograph (Hewlett-Packard) equipped with a variable-wavelength UV–visible absorption detector (flow cell volume: 8 μl). A Model 1046 fluorescence detector (Hewlett-Packard) was alternatively used for the analysis of the LAE commercial mixture. UV-absorption chromatograms were recorded at 291 nm. Excitation and emission wavelengths of 229 and 358 nm were used for the fluorescence detection. A Chemstation HP 3365 Series II data system (Hewlett-Packard) was used for chromatogram acquisition and handling. The samples were injected by a 100-μl syringe (Hamilton) in a manual 7125 injector (Rheodyne) equipped with a 20-μl loop (Rheodyne). The flow-rate was 1.25 ml/min, at controlled room temperature (22 ± 0.5°C).

The separations were carried out using a 125 × 4 mm I.D. LiChrospher 100 RP-18 end-capped 5-μm LiChrocart column (Merck).

The tested mobile phases were prepared by mixing accurately weighed aliquots of HPLC-grade acetonitrile (Baker) and Milli-Q (Millipore) purified water. Both solvents were thermostated at 20°C before being weighed.

The capacity factors were measured at seven different φ values, namely 0.860, 0.880, 0.900, 0.920, 0.940, 0.960 and 0.980. Dead volumes were estimated by injecting 10 μl of Milli-Q water under each mobile phase composition. Mean values of triplicate determinations were used for calculation of the capacity factors.

Normal tetradecanol ($C_{14}OH$) (Aldrich), and the diethyleneglycol ($C_{14}E2$), tetraethyleneglycol ($C_{14}E4$), hexaethyleneglycol ($C_{14}E6$) and octaethyleneglycol ($C_{14}E8$) monotetradecyl ethers (Fluka) were used as standards of individual oligomers. Marlipal 28/100 (Hüls), a mixture of even linear primary fatty alcohol (C_{12}–C_{18}) polyethoxylates with an average number of 10.2 ethoxy units was used as standard of LAE homologues. Stock solutions of these compounds (concentration $\approx$300 μg/ml) were prepared in acetone.

The derivatization reaction was carried out according to the following conditions: 0.5–1-ml aliquots of LAE stock solution were transferred into a screw-capped glass vial and evaporated to dryness under gentle stream of nitrogen. A 10-μl volume of NIC (Aldrich) were then added to the residue. The vial was heated in an oven at 35°C for 30 min. The resulting reaction mixture was finally dissolved in 1 ml acetonitrile [12].

3. Results and discussion

The capacity factors of $C_{14}OH$ and individual C_{14} ethoxymer (i.e. $C_{14}E2$, $C_{14}E4$, $C_{14}E6$ and $C_{14}E8$) NIC derivatives, experimentally determined in the $\varphi = 0.860$–0.980 range, were used to evaluate the constants a and m in Eq. 2 by performing a linear regression calculation. In Fig. 1 the experimental points (i.e. capacity factor logarithms versus mobile phase composition) and the resulting linear regression curves are shown. The correlation coefficients were 0.9965 for $C_{14}E8$, representing the worst case, 0.9995 for $C_{14}E6$ and >0.9995 for all other compounds. A quadratic relationship like Eq. 3 could provide a better fitting of the experimental points and a more accurate prediction of the capacity factors. Nevertheless we did not use this equation because of the high uncertainty in the determination of its coefficients. The results reported below confirmed that a valuable optimization of mobile phase composition for ethoxymer co-elution can be reached using the linear Eq. 2.

Fig. 1 shows that the difference among capacity factors of the tested compounds gradually decreases by increasing φ. Capacity factor logarithms get increasingly closer in the φ range

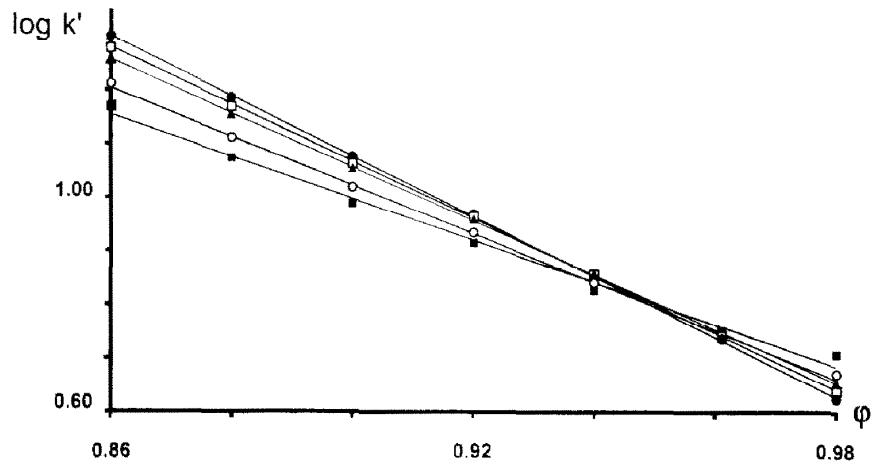

Fig. 1. Capacity factor logarithm versus the fraction of acetonitrile in the mobile phase for NIC derivatives of $C_{14}OH$ (●), $C_{14}E2$ (□), $C_{14}E4$ (▲), $C_{14}E6$ (○) and $C_{14}E8$ (■) in the range $\varphi = 0.86$–0.98. Stationary phase: LiChrospher 100 RP-18 end-capped. Column: LiChrocart 125 × 4 mm I.D. Mobile phase: acetonitrile–water (flow-rate: 1.25 ml/min). Detection: UV absorption at 291 nm.

0.940–0.960, where an inversion of elution order does occur, and then begin again to increase gradually for $\varphi > 0.960$. It follows that it is possible to find the mobile phase composition corresponding to the very near values of the capacity factors. Kudoh [9] carried out a similar chromatographic screening on underivatized LAEs. He found out that LAE ethoxymers co-elution was possible only by using a well determined acetone–water mixture, regardless the length of the LAE hydrophobic chain. Jandera [16] provided a theoretical description for this chromatographic behaviour.

According to the definition [17] of the separation factor (S) as:

$$S = \frac{k'_{max} - k'_{min}}{k'_{max} + k'_{min} + 2} \quad (6)$$

where k'_{max} and k'_{min} represent the capacity factors of the most and least retained compounds among the tested C_{14} LAE ethoxymers, respectively, and known the values of a and m in Eq. 2 for each of them, it is possible to find the value of φ providing the minimum S value. A numerical method was used for this estimation. Based on Eq. 2, the capacity factors at increasing values of φ (implementing step: 0.001) were calculated in the φ range 0.940–0.960. Maximum and minimum values of each k' set were extracted and the corresponding value of S was calculated. Finally the minimum value of S, corresponding to $\varphi = 0.948$, was identified. The results of the optimization are visualized in Figs. 2 and 3. Fig. 2 shows the chromatograms obtained when the derivatized C_{14}OH, -E2, -E4, -E6 and -E8 mixture was eluted under optimal and close-to-optimal mobile phase conditions. It emphasizes the change of elution order with increasing the fraction of acetonitrile in the mobile phase. In particular, the shift of the C_{14}OH peak from the back towards the front of the ethoxymer peaks is evident for φ increasing from 0.940 to 0.960. The RP-HPLC inverse retention order of polyethoxylic oligomers was already reported [18,19] and explained in terms of conformational changes of the polyethoxy chains.

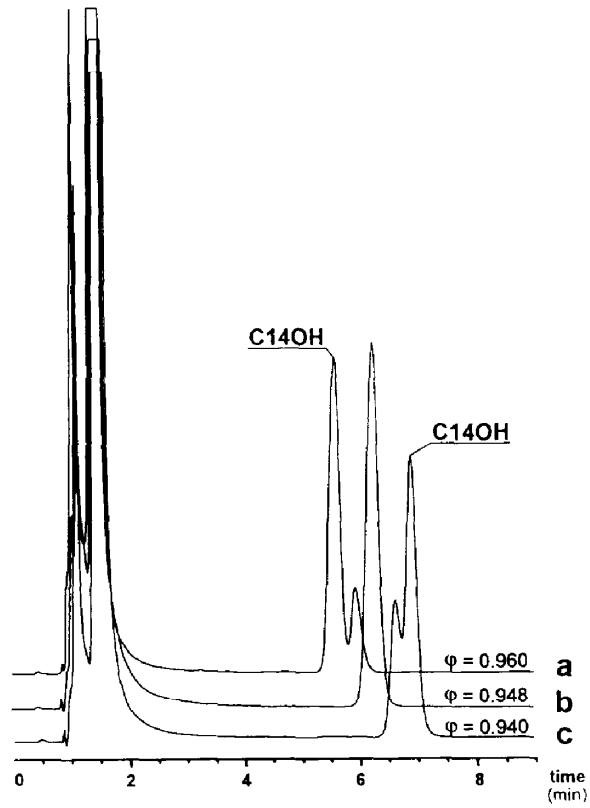

Fig. 2. RP-HPLC chromatograms of NIC derivatives of C_{14}OH, C_{14}E2, C_{14}E4, C_{14}E6 and C_{14}E8 mixture eluting under $\varphi = 0.960$ (a), 0.948 (b) and 0.940 (c) mobile phase conditions. Conditions as in Fig. 1.

The NIC derivatives of a commercial C_{12}–C_{18} LAE mixture, characterized by an average polyethoxy chain length of 10.2 E units, underwent chromatographic separation under the same conditions reported in Fig. 2 for the NIC derivatives of the C_{14}E0–E8 mixture. The corresponding chromatograms are reported in Fig. 3. As is clear from Fig. 3b, the calculated (and experimentally validated) mobile phase composition ($\varphi = 0.948$) yielding optimal co-elution of the NIC derivatives of the C_{14}EO–E8 mixture (Fig. 2a), allowed the effective co-elution, among others, of the C_{14} LAE ethoxymer derivatives whose ethoxylation degree ranged from 0 to over 20 E units. Furthermore, the comparison of Fig. 3b with Fig. 3a and c shows that the optimum value

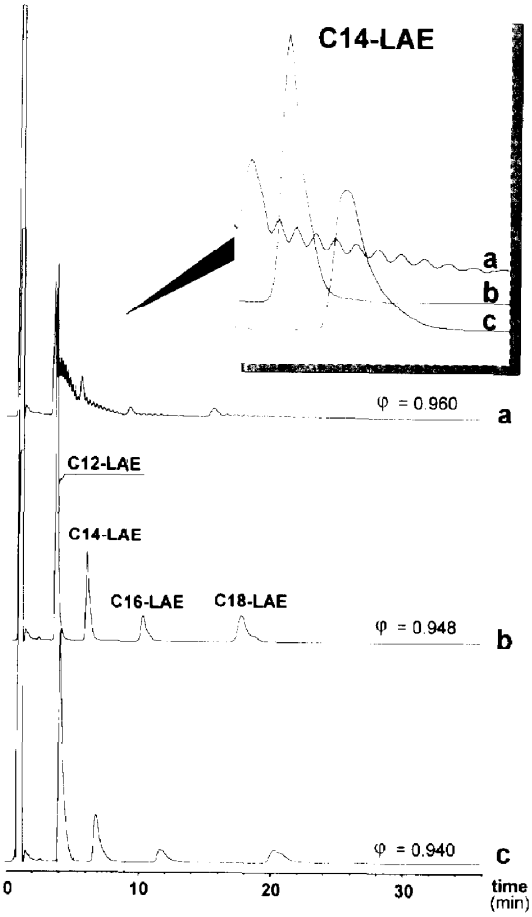

Fig. 3. RP-HPLC chromatograms of the NIC derivatives of Marlipal 28/100 (commercial C_{12}–C_{18} LAEs even homologues mixture; average ethoxylation grade: 10.2) eluting under $\varphi = 0.960$ (a), 0.948 (b) and 0.940 (c). Detection: fluorescence, $\lambda_{ex} = 228$ nm, $\lambda_{em} = 368$ nm. Other conditions as in Fig. 1.

of φ for the co-elution of the C_{14}E0–E8 ethoxymers led also to the most satisfying homologue-by-homologue separation of the derivatized commercial C_{12}–C_{18} LAE mixture. A shift of ± 1% in the mobile phase content of the organic solvent causes significantly worse separations. A partial splitting of the homologue peaks into the corresponding ethoxymers (whose capacity factor increases with the increasing ethoxylation degree) and a partial overlapping of different homologues leading to serious identification and quantitation problems were observed for $\varphi >$ 0.948 (Fig. 3a). A progressive tailing of the homologue peaks and a corresponding decrease of peak heights (i.e. poorer detection and quantitation limits) were obtained after minor increase of the water content (Fig. 3b).

By comparing Fig. 3b with Fig. 3a, a further interesting comment can be drawn. Some higher ethoxymers of each homologue (see magnified details for the C_{14} LAE in Fig. 3) exhibit increased retention by increasing the content of acetonitrile in the mobile phase. This behaviour, responsible for the worsening of the homologue-by-homologue separation for $\varphi > 0.948$, leads to conclude that the retention prediction model based on the linear Eq. 2 is no longer valid for highly ethoxylated LAEs. For these compounds, a model based on the quadratic Eq. 3 is likely to be more suited for the prediction of retention behaviour. In more detail, the higher increase of the quadratic term $+d\varphi^2$ (Eq. 3), compared with that of the linear term $-m\varphi$ (Eq. 2) by increasing φ is claimed to be responsible for the observed increase of retention at the upper limit of the studied φ range.

Fig. 4 visualizes the dependence of log k' of the studied C_{14} LAE ethoxymers on the number of ethoxy units at different fractions of acetonitrile in the mobile phase. The resulting curves suggest that one (or more) curve intersections may occur in correspondence to polyethoxy chain lengths greater than the studied ones. Therefore, the observed increase of retention times of higher ethoxymers of C_{12}–C_{18} LAE homologues by increasing φ (Fig. 3b and a) can be qualitatively foreseen on the basis of the plots reported in Fig. 4. Since these plots are clearly curved, we could use Eq. 5 for the description of the dependence of log k' on n. Unfortunately, an estimation of the constants in Eqs. 3 and 5 based only on the achieved experimental retention data is affected by high uncertainty and practically not feasible. The validation of a retention prediction model based on such equations requires pure standards of highly ethoxylated C_{14} ethoxymers, which are not actually commercially available and difficult to isolate from commercial or laboratory-synthesized LAE mixtures.

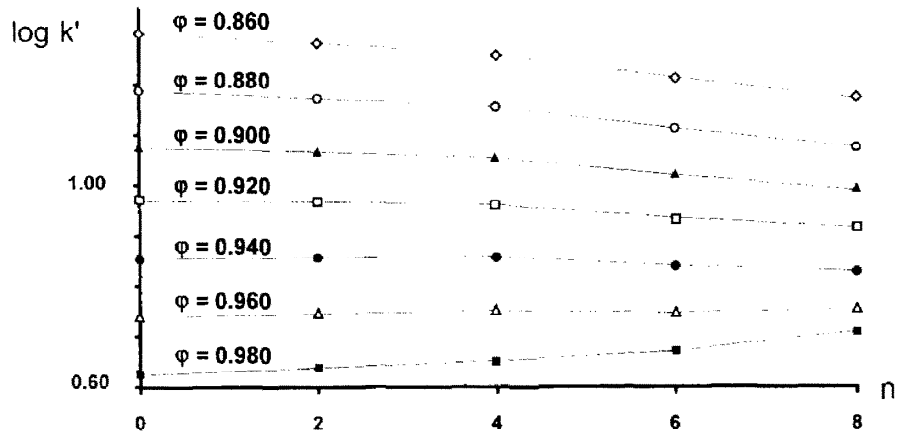

Fig. 4. Capacity factor logarithm versus the number of ethoxy units at different mobile phase composition for NIC derivatives of $C_{14}OH$, $C_{14}E2$, $C_{14}E4$, $C_{14}E6$ and $C_{14}E8$.

4. Conclusions

The feasibility of the homologue-by-homologue separation of NIC derivatives of higher alcohol polyethoxylates was demonstrated. The two most important chromatographic features of the studied system were (a) the change of the retention order in the investigated interval of mobile phase composition and (b) the increasing of the retention of highly ethoxylated LAE molecules with the increasing of the organic solvent amount in the binary mobile phase.

It must be emphasized that the correct preparation of the mobile phase by accurately thermostating, weighing and mixing together the eluent solvents is an essential requisite to obtain the necessary chromatographic reproducibility. The actual most largely certified values of accuracy and precision in solvent proportioning of the commercial multi-channel chromatographic pump systems do not provide an acceptable reproducibility when critical φ values (i.e. those near the isoeluting point) are required, since a slight change of the mobile phase composition leads to a remarkable decrease of both the resolution and the detection sensitivity.

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