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Journal of Chromatography A, 813 (1998) 299–311

JOURNAL OF CHROMATOGRAPHY A

Investigation of chromatographic behaviour of ethoxylated alcohol surfactants in normal-phase and reversed-phase systems using high-performance liquid chromatography–mass spectrometry

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Received 24 April 1997; received in revised form 23 April 1998; accepted 27 April 1998

Abstract

Retention behaviour of ethoxylated alcohols was investigated on octadecyl silica, unmodified silica and aminosilica columns. Good separation according to the alcoholic alkyl length is achieved in reversed-phase systems. The elution order of the oligomers with different numbers of oxyethylene groups depends on the type of the organic solvent (methanol or acetonitrile) and on its concentration in the mobile phase. Different retention behaviour of lower and higher oligomers was observed. The distribution of the oligomers according to the number of oxyethylene units is suppressed, but it overlaps with the peak distribution according to the alkyl length in mobile phases containing high concentrations of acetonitrile. In normal-phase systems, the alkyl length affects the retention much less than the number of oxyethylene units. Better separation than on unmodified silica gel columns can be achieved on a chemically bonded aminosilica column in 2-propanol–*n*-hexane and especially in acetonitrile–water–dichloromethane mobile phases. Possible retention mechanisms in the systems studied are discussed. The retention is strongly affected by solvation of the oxyethylene groups by the mobile phase. The retention factors of higher oligomers with bimodal mass distribution can be described using a simple equation. HPLC–MS with atmospheric pressure chemical ionization allows sensitive detection, easy identification and reconstruction of the chromatographic peaks in chromatograms of complex mixtures of oligomers with different numbers of methylene and oxyethylene groups.

Keywords: Retention behaviour; Surfactants; Ethoxylates; Alcohol ethoxylates

1. Introduction

Polyethoxylated derivatives of aliphatic alcohols, carboxylic acids and phenols are common surfactants widely used in various industrial and household applications as detergents, emulsifiers, lubricants, surface wetting agents, gasoline or cosmetic products additives, enhanced oil recovery agents etc. Non-ionic ethoxylated surfactants became popular since they are more environmental friendly than most other types of surfactants. Commercial products are never pure compounds, but more or less complex mixtures of oligomers with different numbers of oxyethylene (EO) units and often with different alkyl lengths of parent alcohols, acids, etc. The distribution of the individual oligomers depends on the reaction con-

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0021-9673/98/$19.00
PII: S0021-9673(98)00359-8

ditions and on the molar ratio of the components in the reaction mixture. Its determination is important for characterisation of surfactants, as their properties depend both on the structure of the parent compound and on the number of EO units.

The use of gas chromatography for the analysis of surfactants is limited because of low volatility of high oligomers and necessity of derivatisation [1]. Efficient separations of ethoxylated non-ionic surfactants can be achieved by high-performance liquid chromatography (HPLC) both in normal-phase systems on columns packed with unmodified silica gel or with amino-, nitrile- or diol- chemically bonded phases [2–16] and in reversed-phase systems [17–26]. Few reports have been published so far on the systematic investigation of their retention behaviour [27,28].

Individual ethoxylated oligomers are eluted in the order of increasing number of oligomeric oxyethylene units in normal-phase systems. In reversed-phase systems, the order of elution of the oligomers depends on the oligomeric series and on the mobile phase. For example, oligoethyleneglycol phenylethers are eluted in order of increasing numbers of oxyethylene units on an octadecylsilica column [29] and so are oligoethyleneglycol octylphenylethers on a trimethylsilica column in mobile phases comprised of water and methanol [24], whereas oligoethyleneglycol nonylphenylethers are almost unseparated on a C_{18} column in these mobile phases (Fig. 7 in Ref. [30]) and their order of elution is reversed in aqueous acetonitrile [26,31,32], propanol or dioxane [30], like in chromatography on a mixed-mode reversed-phase/ion-exchange column [33].

We have previously studied simultaneous effects of the degree of polymerization, N, and of the concentration of the stronger solvent in binary mobile phases on the retention in non-ionic oligomeric series, both in reversed-phase [30,34], and in normal-phase [35] systems. Recently, we have extended this investigation to the anionic oligomeric surfactants in reversed-phase [36] and normal-phase ion-pair systems [37].

Both in reversed-phase and in normal-phase systems, the logarithms of the retention factor k, change in a linear manner with increasing number of the repeat structural units in the oligomers, N [34]. Commercial ethoxylated alcohols and carboxylic acids contain compounds that differ not only by the number of oxyethylene groups, but also by the lengths (and possibly branching) of the alkyl chains in their parent compounds. If the contribution of each repeat group (methylene and EO) to the retention energy is constant, it should be possible to describe the retention of such co-oligomers with bimodal distribution by Eq. (1)

$$\log k = \log\beta + N_M \log \alpha_M + N_E \log \alpha_E \quad (1)$$

Here, $k=(V_R/V_0-1)$, where V_R is the elution volume and V_0 is the column dead volume, $\log \alpha_M$ and $\log \alpha_E$ characterize the selectivities of separation of adjacent oligomers differing by one repeat M (methylene) or E (oxyethylene) structural unit and $\log \beta$ represents the contribution to the retention by the end group(s) in the oligomeric series. In reversed-phase systems, the logarithms of the retention factors usually decrease in a linear manner with increasing concentration of the organic solvent in the mobile phase, φ:

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

In normal-phase systems with binary organic mobile phases containing two solvents, one less polar (A) and the other more polar (B), the dependence of the retention of co-oligomers on the concentration φ of the solvent B can be often described by a simple equation [35]:

$$\log k = a - m\log \varphi \quad (3)$$

This model was applied to describe the behaviour of ethoxylated nonylphenols on unmodified silica gel and on polar diol-, nitrile- and amino-bonded phases [27,28].

Combinations of Eq. (1) and Eq. (2) or Eq. (3) can be useful for the description of the retention of co-oligomers both in normal-phase and in reversed-phase systems.

It has been found experimentally that the addition of a few percent of water or of a buffer to the mobile phase can improve the selectivity of separation of the individual oligomeric ethoxylated alkylphenols [10,11,15,38–41] on a silica gel or on a bonded amino column. Rissler et al. [42] found improved

selectivity of separation of polyethylene glycol oligomers in gradient-elution chromatography with organic solvents in water using an unmodified silica gel column instead of a C_{18} column.

Co-elution of different oligomers has been used by several groups of workers in so-called ''liquid chromatography at the critical point of adsorption'' (LCCC) for separation of various polymers according to their functionalities or for characterization of block copolymers. The co-elution is attributed to the equilibrium of the adsorption and of the exclusion modes in the chromatographic system. At the ''critical point'' of one block, the other block may be analyzed with respect to its molar mass distribution [43]. This method has been applied to various polymers and oligomers, including polyethylene glycol ethers, for which N_M and N_E represent the number of units in each block. Reversed-phase chromatography with acetonitrile–water [44] or with methanol–water [45] mobile phases was employed for this purpose. Recently, Trathnigg and co-workers have presented theoretical explanation of the behaviour of lower [46] and of higher [47] polyethylene glycol ethers in reversed-phase systems by combined effects of adsorption and size-exclusion.

The identification of the individual compounds in the chromatograms of products containing co-oligomers with two different distribution modes can be difficult if each mode contributes to the retention. In normal-phase systems, more polar oxyethylene groups contribute more strongly to the retention than the length of the alkyl chains, but the effect of the alkyl length may cause peak splitting in some cases. On the other hand, the separation in reversed-phase systems is primarily controlled by the length of the alkyls. In some mobile phases, the separation of the oligomers according to the number of oxyethylene units is suppressed, but the distribution of the peaks depends on the parent compound and on the type and concentrations of the components in the mobile phase. Two different strategies can be adopted for the analysis of complex mixtures of surfactants with bimodal distribution – either the separation into groups with the same number of EO units and with different alkyl lengths, or into groups with the same alkyls, but with different numbers of EO units. Liquid chromatography–mass spectrometry (LC–MS) techniques are particularly useful for the analysis of this type of samples, as they allow mass spectral identification of individual oligomers in the groups separated by HPLC [48,49].

Off-line coupling with fast atom bombardment (FAB) or desorption chemical ionization was originally used for this purpose [50]. Later, direct on-line coupling was possible using new interfaces based on different ionization techniques such as continuous flow FAB [51,52], thermospray [49], or electrospray [53,54]. In the present work, we applied LC–MS with atmospheric pressure chemical ionisation (APCI) for the investigation of retention mechanism of oligomers with bimodal distribution of alkyls and of EO groups in samples of ethoxylated alcohols in normal- and reversed-phase systems.

2. Experimental

2.1. Materials

Methanol, *n*-hexane and dichloromethane were obtained from Baker (Deventer, The Netherlands), acetonitrile and 2-propanol from Labscan (Dublin, Ireland). All solvents were of HPLC grade. Water was doubly distilled in glass (with addition of potassium permanganate and sodium hydrogencarbonate). Glass cartridge columns (150×3 mm I.D.), packed with Separon SGX C_{18}, particle size 7 μm (octadecyl silica), Separon SGX NH_2, 7 μm (aminopropyl silica) and Separon SGX, 5 μm (silica gel), all average pore size 8 nm, were purchased from Tessek, Prague, Czech Republic.

Technical samples of ethoxylated alcohols were obtained as a gift from Bohemiachem (Děčín, Czech Republic): Empilan KBS8 (mixture of ethoxylated dodecyl and tetradecyl alcohols) and Emulan AT9 (mixture of ethoxylated hexadecyl and octadecyl alcohols).

2.2. Chromatographic and mass spectrometric instrumentation

The liquid chromatograph used consisted of a Model 616 pump, a Model 717+ autosampler, a four-channel solvent delivery system (low-pressure gradient system), a thermostatted column compartment, a Model 996 photodiode array detector and a

Millenium chromatography manager (all from Waters, Milford, MA, USA). As the sample compounds do not absorb significantly in the UV region, the outlet from the column was connected either to a Model R 401 refractometric detector (Waters) or directly to a VG Platform quadrupole mass analyser (Micromass, Manchester, UK) with APCI, molecular mass range up to 3000. The signal of the refractometric detector was processed using a Vectra Vl2 personal computer (Hewlett-Packard, Avondale, CA, USA) with a CSW 1.6 chromatographic data station (Data Apex, Prague, Czech Republic).

2.3. Procedures

Mobile phases for isocratic experiments were prepared by pre-mixing appropriate volumes of solvents, filtered through a 0.45-μm Millipore filter prior use and degassed by continuous stripping with helium during the analysis. The samples were prepared by dissolving in the mobile phase in concentrations ca. 1 mg/ml. The column temperature was kept at 40°C (except for two experiments at 65°C) and the flow-rate at 1 ml/min in all experiments. Ten or 20 μl sample volumes were injected into the liquid chromatograph.

Mass spectrometric data were acquired in the range from 35 to 1500 at the scan duration of 1.9 s in the positive-ion APCI mode. A potential of 3.05 kV was applied on the discharge needle. The temperature was held at 500°C in the APCI probe and at 90°C in the ion source. Nitrogen was used as the drying, sheath and nebulising gas. Mild ionization conditions with cone voltage of 10 V were selected to yield mass spectra with little fragmentation.

Each experiment was repeated at least twice. From the retention times, t_R, the retention factors, $k=(t_R/t_0-1)$ were determined. The column hold-up times, t_0, were determined as the elution times of non-retained compounds (methanol and acetonitrile in reversed-phase and *n*-hexane in normal-phase systems) and both t_R and t_0 were corrected for the volumes of the connecting tubing. Each chromatographic peak was attributed to the individual ethoxylated oligomer on the basis of the mass of its $[M+H]^+$ ion in the APCI mass spectrum. Peaks of $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions were identified as the most significant peaks in the mass spectra (Fig. 1), from which the molecular masses and the number of the alcoholic methylene ($-CH_2-$) and oxyethylene ($-CH_2-CH_2-O-$, EO) groups in the individual oligomers were determined with aid of Table 1. With the cone voltage of 10 V, the fragmentation is negligible. From the total ion current (TIC) chromatograms, the chromatograms corresponding to the ethoxylates of the individual alcohols (C_{12}, C_{14}, C_{16} and C_{18}) were reconstructed selecting only the ion currents of the corresponding $[M+H]^+$ ions. The mass spectra revealed also the presence of other ethoxylated alcohols in the samples analysed, but in concentrations of more than two-orders of

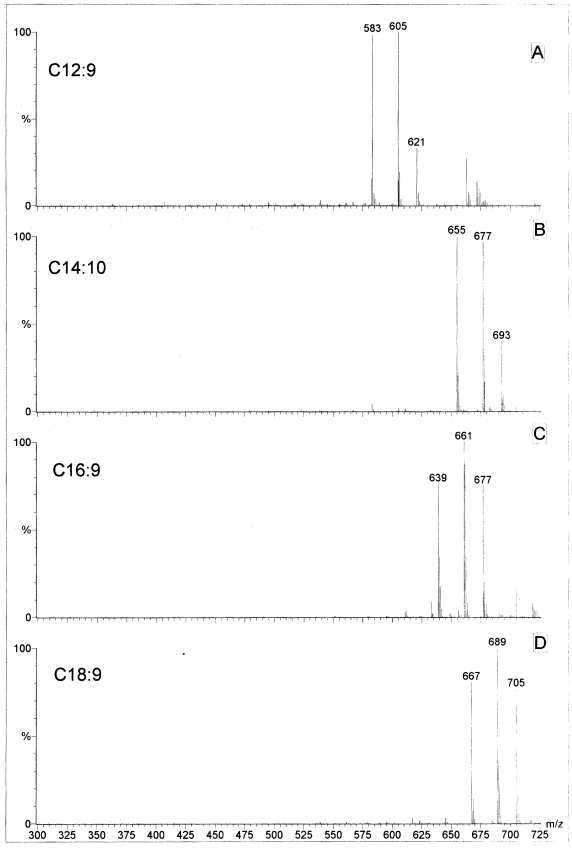

Fig. 1. Examples of the positive-ion APCI mass spectra of several peaks from the chromatograms of Empilan KBS8 and Emulan AT9 on a Separon Amine column, with acetonitrile–water–dichloromethane (69.3:0.7:30) as the mobile phase. Cone voltage = 10 V. Most significant peaks in the mass spectra belong to the ions $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ of ethoxylated alcohols: (A) $C_{12:9}$ (i.e., ethoxylated dodecylalcohol with nine EO units), (B) $C_{14:10}$, (C) $C_{16:9}$ and (D) $C_{18:9}$.

Table 1
Molecular masses of the ethoxylates of aliphatic alcohols with various numbers of oxyethylene units, N_E

N_E	C_{12}	C_{14}	C_{16}	C_{18}
1	230	258	286	314
2	274	302	330	358
3	318	346	374	402
4	362	390	418	446
5	406	434	462	490
6	450	478	506	534
7	494	522	550	578
8	538	566	594	622
9	582	610	638	666
10	626	654	682	710
11	670	698	726	754
12	714	742	770	798
13	758	786	814	842
14	802	830	858	886
15	846	874	902	930

magnitude lower than the concentrations of the main declared alcoholic components.

Multilinear regression analysis using the ADSTAT software (Trilobyte, Prague, Czech Republic) was used to calculate the constants of the dependences of log k on the number of oxyethylene and methylene units (Eq. (1)). The constants of this equation are given in Table 2.

3. Results and discussion

3.1. Chromatography in reversed-phase systems

Retention of the oligomers in the commercial samples containing ethoxylated long-chain alcohols (C_{12}–C_{18}) was measured on a Separon SGX C_{18} column in mobile phases containing acetonitrile or methanol in water. Eq. (1) was found to describe very well the retention of oligomers with eight or more EO units in all mobile phases containing acetonitrile or methanol (except for 100% methanol), with mean error of prediction of $k = 0.5\%$ or less.

In 100% methanol, all oligomers are weakly retained ($k < 1$) and cannot be separated. The retention of the oligomers increases in 90% and in 80% methanol, where the oligomers with different alkyl lengths are well separated. Both the alkyl and the oxyethylene groups increase the retention of ethoxylated oligomers, but the oxyethylene separation selectivity is very low ($\alpha_E = 1.004$ in 90% methanol and $\alpha_E = 1.01$ in 80% methanol) – Table 2. In mobile phases containing 70% or less methanol, the retention is too strong and the time of separation would be impractical. Because of the co-elution of the oligomers with different EO numbers, 90%

Table 2
Parameters of Eq. (1) for reversed-phase and normal-phase systems

Column	EO range	Mobile phase	T (°C)	Log β	Log α_M	Log α_E
1	8–15	a	40	0.545±0.098	0.053±0.005	0.053±0.005
		b	40	−0.922±0.008	0.088±0.001	0.034±0.001
		c	40	−1.152±0.007	0.109±0.0003	0.016±0.0004
		d	40	−1.145±0.005	0.133±0.0003	−0.002±0.0003
		e	40	−1.124±0.003	0.156±0.0001	−0.007±0.0001
		c	65	−1.157±0.038	0.094±0.002	0.012±0.002
		e	65	−1.066±0.008	0.139±0.0002	−0.001±0.0003
		f	40	−1.065±0.005	0.106±0.0003	0.002±0.0002
		g	40	−1.036±0.003	0.151±0.0002	0.006±0.0001
2	2–6	h	40	−0.796±0.335	−0.009±0.025	0.326±0.018
3	2–15	h	40	−0.793±0.04	−0.008±0.003	0.166±0.002
3	2–6	i	40	−0.427±0.028	−0.019±0.002	0.157±0.003
3	7–21	i	40	−0.064±0.013	−0.020±0.001	0.103±0.0004

Columns: 1 = Separon SGX C_{18}, 2 = Separon SGX (silica), 3 = Separon SGX Amine. Subscripts M and E relate to the methylene and oxyethylene selectivity, respectively. Mobile phases: a = 100% acetonitrile, b = 95% acetonitrile in water, c = 90% acetonitrile in water, d = 80% acetonitrile in water, e = 70% acetonitrile in water, f = 90% methanol in water, g = 80% methanol in water, h = 20% 2-propanol in *n*-hexane, i = acetonitrile–dichloromethane–water (69.3:30:0.7).

methanol is the best mobile phase for the separation of the oligomers into groups with different alkyl lengths.

The effect of the concentration of acetonitrile on the separation is complex (Fig. 2). The retention of the individual oligomers decreases as the concentration of acetonitrile increases from 70% to 90%. The methylene selectivity is high, but the oxyethylene selectivity is low in these systems (Table 2). At 40°C, the oligomers with different numbers of EO units are almost completely co-eluted in 80% and in 70% acetonitrile (Fig. 3). In 70% acetonitrile, the retention slightly decreases with increasing number of oxyethylene units ($\alpha_E = 0.998$), but the order of elution is reversed in mobile phases with 90–100% acetonitrile. The effect of a slight variation of a few percent of water on the retention is very dramatic and oxyethylene selectivity increases with increasing concentration of acetonitrile ($\alpha_E = 1.04$ in 90% ACN and $\alpha_E = 1.08$ in 95% ACN) – Table 2, while the methylene selectivity decreases. This causes overlapping distribution of the peaks in the chromatogram. Surprisingly enough, higher oligomers are very strongly retained in 100% acetonitrile where the

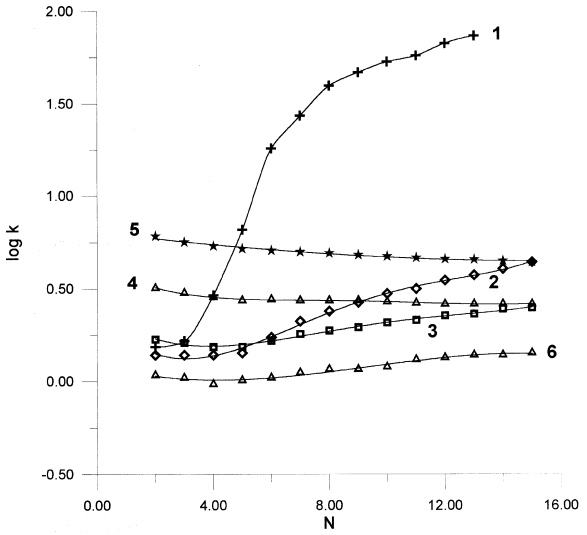

Fig. 2. Dependence of log k on the number of oxyethylene units, N, in ethoxylated dodecylalcohol on a Separon SGX C_{18} column (7 μm, 150×3 mm I.D.) with aqueous acetonitrile as the mobile phases. 1=100% ACN, 2=95% ACN, 3=90% ACN, 4=80% ACN, 5=70% ACN, all at 40°C, 6=90% ACN at 65°C.

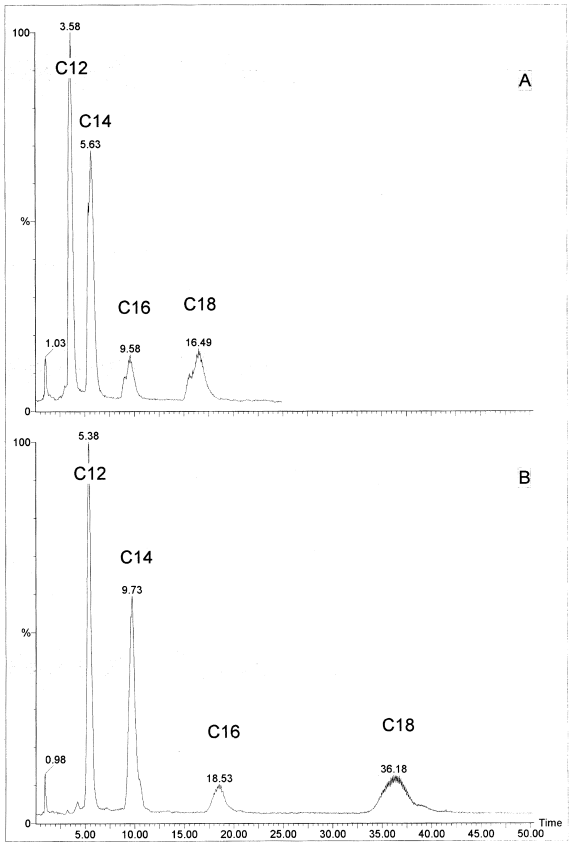

Fig. 3. Separation of a mixed sample of Empilan KBS8 and Emulan AT9 (ethoxylated $C_{12}+C_{14}+C_{16}+C_{18}$ alcohols) on a Separon SGX C_{18} column (7 μm, 150×3 mm I.D.) with 80% (A) and in 70% (B) acetonitrile in water as the mobile phases. Flow-rate 1 ml/min, 40°C, TIC chromatogram. The numbers of the peaks agree with the numbers of the carbon atoms in the alkyls of the oligomers. Time is in min.

oxyethylene selectivity is the same as the methylene selectivity ($\alpha_E = \alpha_M = 1.13$). Fig. 4 illustrates the overlapping peak distribution in 95% acetonitrile as the mobile phase by reconstructed ion current (RIC) chromatograms for the ethoxylated individual alcohols C_{12}–C_{18}, reconstructed from the TIC chromatogram of a mixture containing more than 60 individual compounds. For the clarity sake, only the peaks of the oligomers with three, six, nine and 12 EO units are shown in the RIC chromatograms. The order of elution of the oligomers up to ten EO units in 95% acetonitrile is: $C_{12:2} = C_{12:3} = C_{12:4} < C_{12:5} < C_{12:6} < C_{14:4} < C_{14:3} < C_{12:7} < C_{14:2} = C_{14:5} < C_{12:8} <$

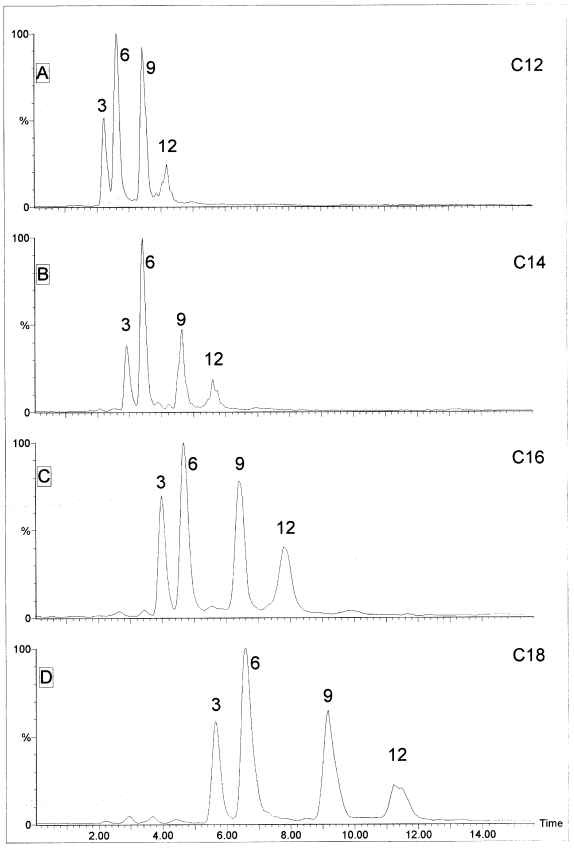

Fig. 4. Separation of a mixed sample of Empilan KBS8 and Emulan AT9 (ethoxylated $C_{12}+C_{14}+C_{16}+C_{18}$ alcohols) on a Separon SGX C_{18} column (7 μm, 150×3 mm I.D.) with 95% acetonitrile in water as the mobile phase. Flow-rate 1 ml/min, 40°C, RIC chromatograms of the ethoxylates derived from different alcohols C_{12} (A), C_{14} (B), C_{16} (C) and C_{18} (D). The numbers of the peaks agree with the numbers of the oxyethylene units in the oligomers (only peaks of the oligomers with 3, 6, 9 and 12 EO units are shown). Time is in min.

$C_{14:6}=C_{12:9}<C_{12:10}<C_{14:7}<C_{16:3}=C_{16:4}<C_{16:2}=C_{16:5}<C_{14:8}<C_{14:9}=C_{16:6}<C_{14:10}<C_{16:7}<C_{18:4}<C_{18:3}<C_{18:5}<C_{16:8}=C_{18:2}<C_{16:9}<C_{18:6}<C_{16:10}<C_{18:7}<C_{18:8}<C_{18:9}<C_{18:10}$, etc. The identification and the reconstruction of the individual chromatographic peaks of such a complex mixture would not be possible without the aid of mass spectral information.

For lower oligomers up to seven oxyethylene units, strong deviations from the validity of Eq. (1) are observed in all mobile phases. In 90% and 95% acetonitrile, the retention first decreases and then increases with increasing number of EO units (Fig. 2).

To explain the retention behaviour in aqueous acetonitrile, the enthalpic and the entropic contributions of the repeat methylene and EO units and of the end groups were calculated from the retention data measured at 40°C and at 65°C in 70% and in 90% acetonitrile, as shown in Table 3 for oligomers with 8–15 EO units. In the two mobile phases, the enthalpic contributions of a methylene group to the retention are positive and are significantly higher than the negative entropic contributions (at 40°C), as a rule in reversed-phase systems. The enthalpic contributions of an EO group are lower than the contributions of a methylene group and are positive in 90% acetonitrile, but negative in 70% acetonitrile. The entropic contribution of an EO group to the retention at 40°C is positive in 70% acetonitrile, but negative in 90% acetonitrile, i.e., opposite to but lower than the enthalpic contributions. The enthalpic and entropic contributions to the retention of strongly polar –OH end groups are strong and negative. The average pore size 8 nm of the octadecylsilica used corresponds exactly to the length of the stretched largest oligomer – ethoxylated C_{18} alcohol with 15 EO units. Because the oligomers are likely to be in a folded conformation, the pore volume should be readily accessible to all oligomers and size-exclusion effects (if any) are probably not large enough to explain the retention behaviour observed.

Combined effects of the polarity and of the solvation seem more likely to explain the experimental retention behaviour. It was shown earlier that the separation selectivity for a repeat group generally decreases with decreasing polarity of the mobile phase, i.e., with increasing concentration of the organic solvent in the mobile phase [30]. This is in agreement with the experimental methylene and EO selectivities in aqueous methanol and with the experimental methylene selectivity in aqueous acetonitrile. Contrary to this rule, the experimental EO selectivity decreases as the concentration of acetonitrile in the mobile phase decreases and eventually the elution order is reversed in mobile phases with 80% or less acetonitrile. This behaviour can be possibly explained by different solvation effects in methanol–water and in acetonitrile–water mobile phases. The

Table 3
Enthalpic and entropic contributions to the retention of ethoxylated alcohols C_{12}–C_{18} with 8–15 oxyethylene groups in reversed-phase systems with acetonitrile–water mobile phases

		Mobile phase	
		Acetonitrile–water (70:30)	Acetonitrile–water (90:10)
$-\Delta(\Delta H^0)$	Me	1.17	1.23
(kJ mol^{-1})	EO	−0.48	0.32
	EG	−1.97	−0.10
$\Delta(\Delta S^0)$	Me	−0.75	−1.54
(J mol^{-1} K^{-1})	EO	1.36	−0.71
	EG	−12.67	−23.48
$\Delta(\Delta S^0)T$ at 40°C	Me	−0.22	−0.48
(kJ mol^{-1})	EO	0.43	−0.22
	EG	−3.97	−7.35

Column: Separon SGX C_{18}. $-\Delta H^0$ and ΔS^0 determined from the experimental retention factors, k, measured at 40°C and 65°C, using the equation: $\log k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \log \phi$, where T = temperature in Kelvins, R = gas constant = 8.314 J mol^{-1} K^{-1}, $\phi = V_S/V_M$ = phase ratio, i.e., the ratio of the volume of the stationary (V_S = 0.41 ml) and of the mobile (V_M = 0.87 ml) phases in the column. $-\Delta(\Delta H^0)$ and $\Delta(\Delta S^0)$ = contributions of the repeat $-CH_2-$ (Me) and $-CH_2CH_2O-$ (EO) units and of the end groups (EGs). The contributions of the individual groups were determined by linear regression of the dependencies of ΔS^0 and of ΔH^0 on the number of the methylene and of the EO units.

oxyethylene groups are subject to proton–acceptor interactions with –OH groups of water and methanol, but such interactions are not possible with acetonitrile, which is a poorer solvent with respect to the EO groups. Consequently, addition of water to pure acetonitrile improves the solvation, increases the entropic contribution but decreases dramatically the enthalpic contribution and consequently the retention, especially of the oligomers with higher number of EO units. The coelution of the oligomers with different numbers of EO groups in 80% acetonitrile occurs not only because of the equilibration of the enthalpic and the entropic contributions of the EO groups to the retention, but both contributions are close to zero in this mobile phase.

The deviations from the regular retention behaviour of lower oligomers can be possibly explained by different composition of the liquid phase contacting the EO groups close to and more distant from the alkyl chain. The non-polar alkyls C_{12}–C_{18} in the molecules of the oligomers can penetrate in between the bonded non-polar octadecyl chains of the fur-like stationary phase and the oxyethylene chains are oriented towards the bulk polar mobile phase. The length of the alkyl chains in the polyethyleneglycol ethers studied practically rules out the possibility that some of the EO groups could come into contact with unreacted silanol groups on the surface of the packing material. However, less polar organic solvents are preferentially adsorbed on to the surface of the stationary phase and may form several adsorbed molecular interface layers more rich in the organic solvent than the bulk mixed aqueous–organic mobile phase [55]. This means that the EO groups close to the ethoxylate alkyl chains are consequently close to the surface of the stationary phase and come into contact with mobile phase more rich in the organic solvent than more distant EO groups. As the contribution of an EO group to the retention increases with increasing concentration of acetonitrile (Table 2), the lower oligomers surrounded with more concentrated acetonitrile are more strongly retained than it would be expected from the linear dependences of log k on the number of EO groups in higher oligomers (Fig. 2). The S-shape form of the plot for 100% (and – to a lesser extent – for 95% and 90%) acetonitrile in Fig. 2 can be possibly explained by a more rigid orientation of lower oligomers towards the surface of the stationary phase. For the oligomers with six or less EO units, the entropy of retention is approxi-

mately constant, for higher oligomers it slightly increases with increasing number of oxyethylene units.

3.2. Chromatography in normal-phase systems

We investigated chromatographic behaviour of ethoxylated alcohols in the systems used earlier for the separation of ethoxylated alkylphenols in normal-phase systems [37,38], i.e., on silica gel and amino-propyl silica columns with 2-propanol in *n*-hexane and aqueous acetonitrile in dichloromethane as the mobile phases. In all tested systems, Eq. (1) was found to describe well simultaneous effects of the number of methylene and of EO groups on the retention factors, with similar main error in the *k* values as in reversed-phase systems. The methylene and the EO selectivity parameters are listed in Table 2.

On a Separon SGX silica gel column with 20% 2-propanol in *n*-hexane, the separation of the individual oligomers with different numbers of oxyethylene units is complete, but only six oligomers could be separated in 30 min, as the separation selectivity (log α_E, Table 2) is much too high. The oligomers are eluted in the order of increasing number of EO units, but unlike to reversed-phase systems, the retention decreases with increasing alkyl length of the parent alcohol. The effect of the number of alcoholic methylene groups on the retention is much less significant than the effect of the number of EO units and it results only in splitted peaks of higher oligomers.

With 20% 2-propanol in *n*-hexane as the mobile phase, the retention factors, *k*, on a Separon SGX Amine column increase regularly with the number of oxyethylene units, but the EO selectivity (log α_E) is half the value on the unmodified silica gel column (Table 2) and the ethoxylated alcohols are much less retained, which makes the separation on the Amine column more practical. The alkyl length has little effect on the retention (Fig. 5A). Eq. (3) describes well the effect of the concentration of 2-propanol in the mobile phase on the retention (Fig. 6A). Both the intercept *a* and the slope *m* of the concentration dependences of retention factors (Eq. (3)) increase in a linear manner with the number of EO units in the oligomer (positive value of m_1, Table 4), which

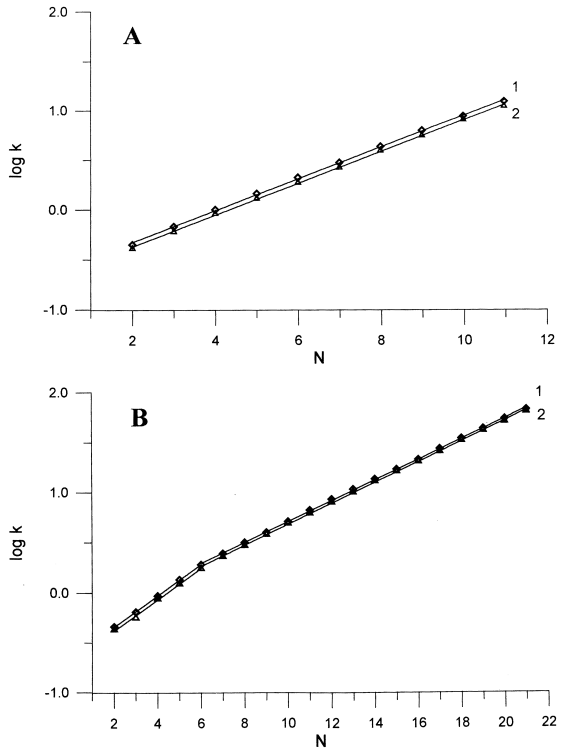

Fig. 5. Dependence of log *k* on the number of oxyethylene units, *N*, in ethoxylated alcohols C_{12} (1) and C_{14} (2) in Empilan KBS8 on a Separon SGX NH_2 column (7 μm, 150×3 mm I.D.) with: (A) hexane–2-propanol (80:20), (B) acetonitrile–water–dichloromethane (69.3:0.7:30) as the mobile phases.

means that both the separation selectivity and the time of separation increase at lower concentrations of 2-propanol.

Finally, retention of ethoxylated alcohols was studied in mobile phases containing aqueous acetonitrile in dichloromethane, which provides good separation of ethoxylated nonylphenols [38]. The TIC chromatogram of Emulan AT9 (Fig. 7A) is compared with the reconstructed chromatograms of the ethoxylated C_{16} and C_{18} alcohols (Fig. 7B,C) with acetonitrile–water–dichloromethane (69.3:0.7:30) as the mobile phase. The retention slightly decreases with increasing alkyl lengths, like in the 2-propanol–*n*-hexane mobile phase (Fig. 5B). In the TIC chromatogram, the distribution according to the alkyl chain length would not be apparent without the MS identification.

Linear increase of log *k* with increasing number of

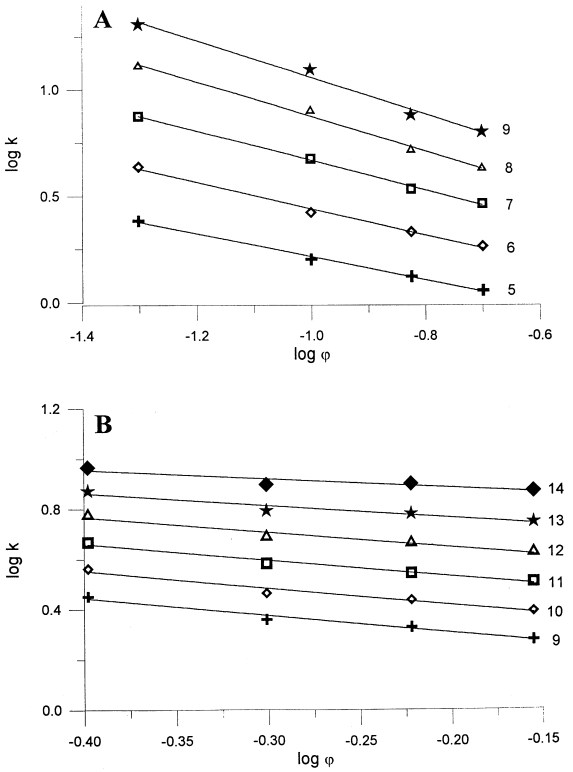

Fig. 6. Dependence of the retention factors, *k*, of ethoxylated tetradecyl alcohol on the concentration φ (%, v/v, $\cdot 10^{-2}$) of: (A) 2-propanol in hexane, (B) aqueous acetonitrile (with 1% of water) in dichloromethane. Column: Separon SGX NH_2 (7 μm, 150×3 mm I.D.). The numbers of plots agree with the numbers of oxyethylene units in the oligomers.

EO units was observed, but unlike the propanol–hexane mobile phase (Fig. 5A), two linear parts of the plots with different slopes (oligomeric selectivities, log α) can be clearly distinguished, with the change in the slope occurring at six oxyethylene units (Fig. 5B). The breaks on the log *k* versus *N* plots can be possibly explained by different solvation and conformation of the longer and shorter oxyethylene chains in mobile phases containing small amounts of water. Water becomes preferentially adsorbed and may form a layer on the polar surface of the bonded aminosilica phase. Unlike to reversed-phase systems, the oligomers are adsorbed by more polar oxyethylene parts of their molecules, with alkyl chains oriented from the surface of the adsorbent towards the bulk mobile phase. As water solvates better the EO units than either acetonitrile or dichloromethane, the oligomers with lower number of EO units stick more close to the adsorbent surface, are better solvated and more strongly retained than the oligomers with longer oxyethylene chains. This results in a higher EO selectivity of lower oligomers, close to the selectivity in 20% propanol in *n*-hexane mobile phase, where little difference in solvation of EO units with propanol can be expected both close to the adsorbent surface and in the bulk mobile phase (Table 2). Practical consequence of a decreased EO selectivity for higher oligomers is that the separation of a larger number of oligomers is feasible in reasonable time than with propanol–hexane mobile phases.

Table 4
Parameters *a*, *m* of Eq. (3) describing the retention of ethoxylated dodecyl alcohol (C_{12}) with various numbers of oxyethylene units, *N*, in normal-phase chromatographic systems

Acetonitrile(+1% water)–dichloromethane				2-Propanol–*n*-hexane			
N_E	*a*	*m*	*R*	N_E	*a*	*m*	*R*
9	0.1696	0.6884	0.9729	5	−0.3236	0.5389	0.9956
10	0.2821	0.6791	0.9637	6	−0.1804	0.6211	0.9940
11	0.4073	0.6313	0.9771	7	−0.0259	0.6930	0.9977
12	0.5300	0.5901	0.9581	8	−0.0558	0.8181	0.9931
13	0.6708	0.4769	0.9281	9	0.1938	0.8646	0.9863
	$a_0=-1.009$	$a_1=0.1294$	0.9977		$a_0=-1.070$	$a_1=0.1489$	0.9996
	$m_0=1.3472$	$m_1=-0.0677$	0.8932		$m_0=0.1544$	$m_1=0.0772$	0.9984

Column: Separon SGX Amine, 7 μm, 150×3 mm I.D., 40°C. *R*=correlation coefficient.
a_0, a_1, m_0, m_1 are parameters of the dependencies of *a* and *m* on the number of EO units, N_E: $a=a_0+a_1N_E$, $m=m_0+m_1N_E$.

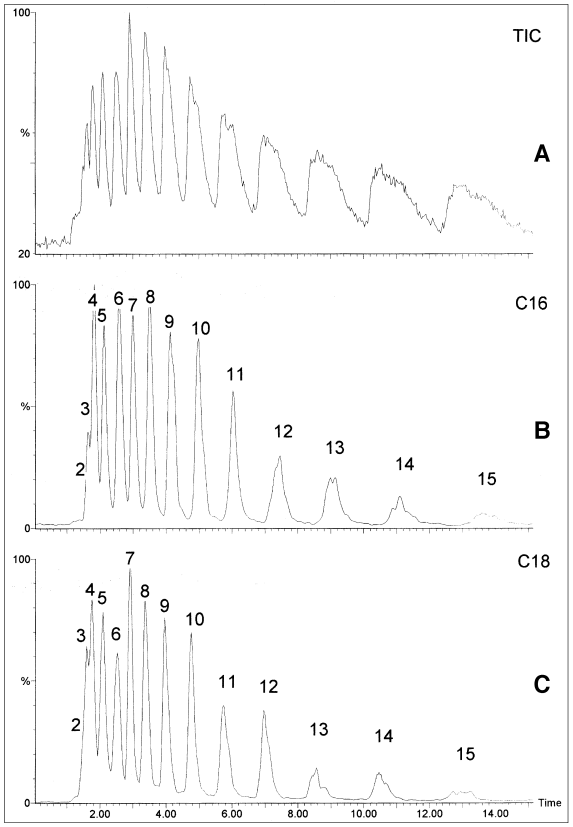

Fig. 7. Separation of ethoxylated alcohols in Emulan AT9 on a Separon SGX NH_2 column (7 μm, 150×3 mm I.D.) with acetonitrile–water–dichloromethane (69.3:0.7:30) as the mobile phase. Flow-rate 1 ml/min, 40°C. The numbers of peaks agree with the numbers of the oxyethylene units in the oligomers. Time is in min. (A) TIC chromatogram, (B) RIC chromatogram of $[M+H]^+$ ions of C_{16} alcohol ethoxylates, (C) RIC chromatogram of $[M+H]^+$ ions of C_{18} alcohol ethoxylates.

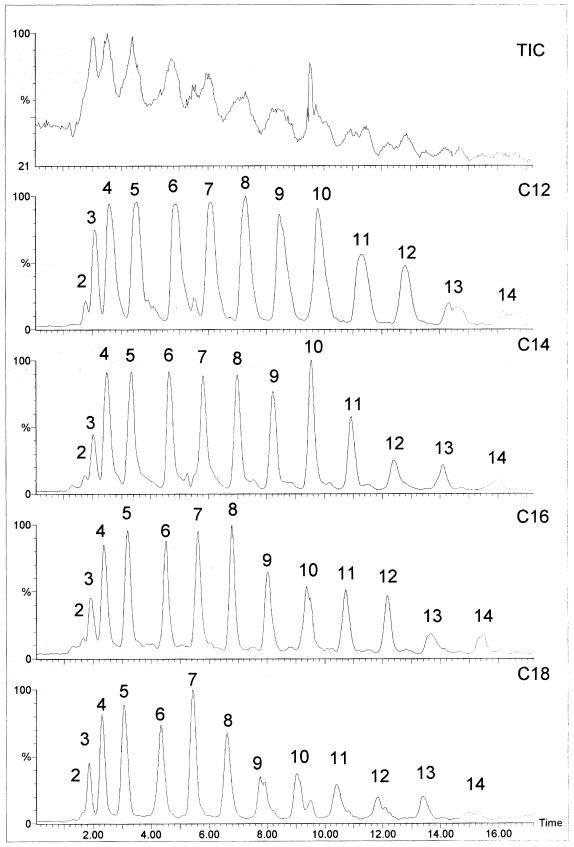

Fig. 8. Separation of ethoxylated alcohols in a mixed sample of Empilan KBS8 and Emulan AT9 (ethoxylated $C_{12}+C_{14}+C_{16}+C_{18}$ alcohols) on a Separon SGX NH_2 column (7 μm, 150×3 mm I.D.) using gradient elution from 40% to 90% aqueous acetonitrile (with 1% water) in dichloromethane in 10 min. Flow-rate 1 ml/min, 40°C. TIC chromatogram and RIC chromatograms of $[M+H]^+$ ions of C_{12}–C_{18} alcohol ethoxylates. The numbers of peaks agree with the numbers of the oxyethylene units in the oligomers. Time is in min.

As shown in Fig. 6B, the effect of the concentration of aqueous acetonitrile on the retention can be adequately described by Eq. (3). The dependence of the parameters *a*, *m* of this equation is linear, but the slope *m* decreases with increasing number of EO units, in contrast to the behaviour in 2-propanol–*n*-hexane mobile phases (negative value of the parameter m_1). This means that the oligomeric selectivity is higher at higher concentrations of aqueous acetonitrile in the mobile phase (Table 4), which is favourable for practical separation purposes, as it makes possible better separation in a shorter time of analysis.

Better separation of a broader range of oligomers than under isocratic conditions can be achieved using gradient elution, as it is demonstrated by reconstructed chromatograms of the individual ethoxylated alcohols C_{12}, C_{14}, C_{16} and C_{18} in a mixed sample of Empilan KBS 8 and Emulan AT9 using a gradient of increasing concentration of acetonitrile in dichloromethane in Fig. 8. Fourteen oligomers are resolved in ca. 15 min.

4. Conclusions

In reversed-phase systems, the samples of ethoxylated alcohols are well separated according to the alcoholic alkyl lengths. The separation according to the number of oxyethylene units is generally suppressed, which makes possible the determination of the concentration ratios of the ethoxylates derived from different alcohols. However, in mobile phases with low concentrations of water in acetonitrile necessary to accomplish the separation in a reasonable time, the oxyethylene units contribute to the retention and the peak distribution according to the alkyl lengths overlaps the distribution according to the number of oxyethylene units. Consequently, aqueous methanol is better suited than aqueous acetonitrile for the separation of ethoxylated alcohols into groups with different alkyl lengths.

For normal-phase separations of ethoxylated alcohols, columns with chemically bonded aminophases offer shorter separation times than columns with unmodified silica gel. With 2-propanol–*n*-hexane mobile phases, separation of the oligomers into groups according to the number of oxyethylene groups is possible. In acetonitrile–water–dichloromethane mobile phases, better separation of the oligomers according to the distribution of the oxyethylene units can be achieved, especially when gradient elution is employed.

The dependence of the retention on the number of both methylene and oxyethylene units can be described by a simple equation, in agreement with the assumption of independent contributions of the two types of repeat units to the retention. However, with mobile phases containing low amounts of water in acetonitrile or in acetonitrile–dichloromethane mobile phases, this dependence applies for higher oligomers only, both on octadecylsilica and on aminosilica bonded phase columns. The order of elution of the oxyethylene oligomers and the deviations observed for lower oligomers can be explained by solvation effects of the oxyethylene groups. The retention mechanism suggested is supported by the experimental values of enthalpic and entropic contributions to the retention. Similar effects, which may result into surprisingly ''irregular'' retention behaviour, are likely to occur also with other oligomers containing more than one repeat structural groups with different polarities.

The application of the HPLC–MS technique with atmospheric pressure ionization is very helpful for the separation of oligomers with bimodal distribution according to the number of oxyethylene units and to the alcoholic alkyl length, as it makes easy unambiguous identification of the peaks separated according to one of the distribution modes, reconstruction of the chromatograms of the ethoxylates derived from the individual alcohols and determination of the distribution of the oligomers. This technique can be easily applied in connection with gradient elution of ethoxylates that do not absorb in the UV region.

Acknowledgements

This publication is based on work under Project No. 203/98/0598 sponsored by the Grant Agency of Czech Republic and by subvention from VS-96068 MŠMT-ČR. G.T. is grateful to a NATO fellowship granted from the Greek Ministry of Economics (Reg. No. 7421/ΔΟΟ 386), for the financial support of his study. The authors thank Mr. Petr Zderadička for technical assistance with some experiments.

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