

Dedicated to Professor Victor-Emanuel Sahini
on the occasion of his 80th anniversary

THE RETENTION BEHAVIOR OF ETHOXYLATED NONIONIC SURFACTANTS IN REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SYSTEMS

Marieta BALCAN,^{a*} Dan F. ANGHEL,^a Dan DONESCU^b and Valerică RAICU^c

^a Department of Colloids, “Ilie Murgulescu” Institute of Physical Chemistry,

Spl. Independentei 202, 060021, Post Office 12, P. O. Box 194, Bucharest, Roumania

^b Researcher Center for Polymer and Plastic Materials, Spl. Independentei 202, 060021 Bucharest, Roumania

^c Department of Physics, University of Wisconsin-Milwaukee, Milwaukee, WI 53201-0413, USA

Received November 17, 2006

The retention behavior of homogeneously ethoxylated alcohols (C_nE_8) and nonylphenols (NPE_m) has been investigated by reversed-phase high performance liquid chromatography (RPLC) on octadecyl-silica columns with methanol-water and acetonitrile-water eluents. The effects of mobile phase composition, the alkyl chain length (n) and the EO group number (m) of the surfactants have been considered. The retention plots ($\log k'$ vs. organic solvent concentration) of C_nE_8 were linear with methanol-water and acetonitrile-water eluents while those of NPE_m were linear only with methanol-water. For the acetonitrile-water eluent, the deviation from linearity increases with the ethoxylation degree. In both elution systems, the retention plots had a turning point at about 20 % (vol.) water in the eluent. To explain the irregularities observed in the behavior of NPE_m , the variation of molar refraction and dielectric constant of the hydro-organic mixtures was investigated. For both elution systems, the excess molar refraction (R^E) was negative on the whole composition range. The values were more negative for acetonitrile than for methanol, indicating a stronger modification of the polar interactions between the water molecules and those of the former organic modifier. The values of the excess dielectric constant (ϵ^E), were negative except those in the water-rich region of the methanol-water mixture. For acetonitrile-water, two regions of positive values bordered the negative ϵ^E data. The changes in the physical properties of solutions were related to the investigated retention behavior of the surfactants. The retention mechanism involved in the studied systems was also discussed.

INTRODUCTION

Nonionic ethoxylated surfactants are widely used in various industrial and household applications as detergents, emulsifiers, wetting agents, lubricants, cosmetic additives, enhanced oil recovery agents, etc. They are amphiphilic substances having in molecule a non-polar alkyl or alkyl-aryl part and a polar poly(ethylene oxide) (PEO) moiety. The properties and applications of these compounds greatly depend on the equilibrium between the hydrophobic and the hydrophilic segments of the molecule. In contrast with commercial products that are complex mixtures having similar hydrophobic part but

different PEO chain lengths, the homogeneous ethoxylated surfactants have well determined number of ethylene oxide units in the molecule. In aqueous solution, the critical micelle concentration (CMC) and the head-group area of the formers decrease linearly with the width of ethoxymers distribution.¹ The surface tension at the CMC for technical surfactants is lower than for pure oligomer species, due to the selective adsorption of the shorter ethoxymers at the surface. There are situations, especially in the oil-water systems, when each molecular species manifest individual adsorption tendencies leading to ethoxymers fractionation, which seriously affects the phase behavior and the partition.^{2,3} Therefore, it is

* Corresponding author: mbalcan@icf.ro

important to investigate and compare the behavior of homogeneous and polydisperse ethoxylated compounds under these conditions.

High-performance liquid chromatography (HPLC) is particularly useful for characterization of ethoxylated nonionic surfactants.⁴ The normal-phase liquid chromatography (NPLC) allows separation of surfactants upon the PEO chain length⁵⁻⁸ whereas, the reversed-phase liquid chromatography (RPLC) gives information on the hydrophobic tail.^{7,8} Using proper combinations of stationary and mobile phases, the separation of the ethylene oxide (EO) oligomers of the surfactants was also achieved in RPLC.⁹⁻¹¹

In reversed-phase chromatographic systems with alkyl-bonded stationary phases and hydro-organic eluents, the retention of ethoxymers may increase with the EO group number, decrease or remain unchanged depending on the organic modifier and its mixing ratio with water.^{8,9,12-14} The retention increases with temperature being different from that of other organic polymers.¹⁵⁻¹⁷ To explain these irregularities and the inversion point of elution several attempts have been made.^{8,9,17} However, it seems that ethoxylates retention is difficult to elucidate, because it depends on their interactions with both the stationary and the mobile phase. Besides that, the stationary phase, the organic modifier and its concentration, the temperature, the length of the alkyl and PEO chains also affect the surfactant behavior. Therefore, the retention mechanism of ethoxylated surfactants in RP-HPLC is still a topic of debate.

In our previous studies we demonstrated that the hydrophobic interactions govern the ethoxylated surfactants retention on reversed-phase systems with alkyl-silica columns and methanol-water eluents.^{13,14} We found out good correlations between the retention data and the hydrophobicity parameters such as the hydrophile-lipophile balance (HLB) and the oil/water partition coefficient (K_{ow}). The enthalpy-entropy compensation results indicated that the mechanism of retention is similar for homogeneous^{18,19} and polydisperse ethoxylated surfactants.²⁰ In the behavior of polydisperse ethoxylated nonylphenols on the octadecyl-silica column, we have observed differences when methanol was replaced with acetonitrile in the mobile phase.²¹ The change of surface tension (γ) as a function of composition of the hydro-organic eluents was not adequate to explain the irregularities in the retention of ethoxylates. The viscosity deviation ($\Delta\eta$) indicated

that acetonitrile interacts more feebly with water than methanol, and affects the solvation of the stationary phase and its interaction with the ethoxylates.²¹

The objective of this paper is to extend the above mentioned investigations to homogeneous ethoxylated fatty alcohols and nonylphenols. The effect of hydrophobic chain length of the homogeneously ethoxylated fatty alcohols (C_nE_8) and that of the EO group number of homogeneously ethoxylated nonylphenols (NPE_m) will be evaluated. The surfactants will be eluted with methanol-water and acetonitrile-water mixtures and the results compared. To get more insight in surfactant retention, measurements of molar refraction and dielectric constant of the eluents will be done and related to the observed irregularities in surfactant retention. Finally, considerations about the retention mechanism of these compounds will be put forward.

EXPERIMENTAL PART

Chemicals

Homogeneously ethoxylated fatty alcohols (C_nE_m) with different alkyl chain lengths ($n = 10, 12, 14,$ and 16) and the same PEO moiety ($m = 8$), and homogeneously ethoxylated nonylphenols (NPE_m , $m = 7 - 15$) with the same hydrophobic part and different PEO chain lengths were purchased from: Fluka Chemie AG, Buchs, Switzerland ($C_{10}E_8$ and $C_{14}E_8$) and Nikko Chemicals Co. Ltd., Tokyo, Japan ($C_{12}E_8$ and $C_{16}E_8$). They were used without purification. To be detectable by UV, they were derivatized by reacting with phenyl isocyanate.^{5,14} Ethoxylated nonylphenols with definite grade of ethoxylation were obtained as previously reported.⁷ The methanol and the acetonitrile were HPLC-grade reagents. Double distilled water having an electrical conductivity lower than $1.5 \mu\text{S}/\text{cm}$ was employed. To remove particulate matter, the water was passed through 4612 Acrodisc filters (Pall Gelman Sciences, Vienna, Austria).

Methods

Chromatography

The chromatographic measurements were carried out as reported elsewhere,^{7,13,14} and on a modular Varian HPLC system made of Prostar 240 quaternary pump, Prostar 410 autosampler, Prostar 335 PDA diode array detector and operated by Workstation Software. The mobile phase consisted of various hydro-organic mixtures with methanol and acetonitrile as organic modifier, and the flow rate was $1\text{mL}/\text{min}$. The running solutions for HPLC had a concentration of $10\text{ mg}/\text{mL}$. They were prepared in methanol-water and acetonitrile-water mixtures (4:1, v/v) and stored at 4°C . The injected volume was of $10\text{ }\mu\text{L}$.

The retention time (t_r) of every surfactant was obtained as a mean of at least three individual determinations. For each chromatographic system, the dead time (t_0) was measured with

deuterium oxide. The capacity factor (k') was calculated according to $k' = (t_r - t_0)/t_0$.

Molar refraction

The molar refractions (R_m) of the eluents were determined from their refractive index (n) and density (ρ) values according to Lorenz-Lorentz equation:²²

$$R_m = [(n^2 - 1) M] / [n^2 + 2] \rho \quad (1)$$

where, M is the average molar mass of the mixture.

Densities, ρ , of the pure liquids and their binary mixtures were measured at 40 °C by using a pycnometer having a bulb volume of 10 cm³. An average of triplicate measurements was considered, and these were reproducible within ± 0.0001 g cm⁻³. The refractive indices, n , for the sodium-D line were measured with an Abbé refractometer, at 40 °C. The choice of this temperature was related to the working temperature of the chromatographic system. The reproducibility in the refractive index data was within $\pm 0.2\%$.

The excess molar refraction (R^E) of solutions was calculated from the Lorenz-Lorentz mixing rule:²³

$$R^E = R_m - \phi R_1 - (1 - \phi) R_2 \quad (2)$$

where, R_m is the molar refraction of the mixture determined by using equation (1), R_1 and R_2 are the molar refraction of pure water and of organic solvent respectively, and ϕ is the volume fraction of water in the hydro-organic mixture.

Dielectric measurements

Measurements of electrical capacitance at fixed frequency (10 MHz) of hydro-organic eluents were carried out with a Hewlett Packard impedance-analyzer, model 4194 A. The measuring cell was an open-ended coaxial line previously described.²⁴ The dielectric constant is related to the measured capacitance through equation (3):

$$\epsilon = C/k \quad (3)$$

The cell constant ($k = 0.00235$ m) was determined by calibration with water, for which the permittivity is known. Final permittivity data were obtained by averaging at least 200 values for each sample. The standard deviation of data was less than 1%. The values of excess dielectric constant, ϵ^E , of the hydro-organic solutions were calculated using Decroocq's simplified formula:²⁵

$$\epsilon^E = \epsilon_m - \phi \epsilon_1 + (1 - \phi) \epsilon_2 \quad (4)$$

where, ϵ_m is the measured dielectric constant of binary mixture, ϵ_1 and ϵ_2 are the dielectric constants of pure water and organic solvent, and ϕ is the volume fraction of water in the hydro-organic mixture.

RESULTS AND DISCUSSION

Figure 1 illustrates the semilog plots of the capacity factor of ethoxylated fatty alcohols (C_nE_8) as a function of methanol concentration. For surfactants having the same PEO chain but different methylene group number, the retention increases with the length of the alkyl chain. Within the investigated range of eluent composition, the retention increases linearly for all the surfactants with the water content of the mobile phase. Figure

2 also shows a linear dependencies of the C_nE_8 retention with acetonitrile-water mixtures as mobile phases. Our $\log k'$ values vs. eluent composition obey equation (5) previously proposed by Snyder:²⁶

$$\log k' = \log k_w - b \cdot \phi \quad (5)$$

where, ϕ is the organic modifier concentration in the eluent, and the intercept, k_w , is the capacity factor with pure water as standard eluent. The slope, b , stands for the variation of the retention factor when ϕ changes with 1% (vol.) and characterizes not only the strength of the eluent but also the interactions between eluite, and stationary and mobile phases. Table 1 summarizes the obtained parameters together with the correlation coefficients, r . The negative slopes indicate the decrease of retention by increasing the less-polar solvent content of the eluent. In absolute value, the slopes increase with the alkyl chain of the surfactant as previously noticed for ethoxylated fatty alcohols in RPLC.^{14,27}

One may observe that for the same surfactant, at a given eluent composition, the retention parameter, $\log k'$, is smaller when acetonitrile is used as organic modifier. This can be explained by a smaller polarity of acetonitrile and hence a higher solvent strength in comparison with methanol.²⁸

For each elution system, $\log k_w$ increases with the alkyl chain length of the surfactant. The linear relationships between $\log k_w$ and the alkyl carbon number of the surfactants attest the important information content of $\log k_w$ about the hydrophobic character of eluite. Therefore, it may be considered as a reliable parameter of the hydrophobicity in RPLC. The slope represents the contribution of the alkyl methylene group to the $\log k_w$, and allows calculation of the methylenic increment of the standard free energy ($\Delta\Delta G^\circ$) of transfer from water as mobile phase to the non-polar stationary phase. The $\Delta\Delta G^\circ$ value obtained in the present study for $C_{10-16}E_8$ series on octadecyl-silica column and methanol-water eluent is equal to -2.93 kJ/mol. It is quite close to that of -2.53 kJ/mol for homogeneously ethoxylated fatty alcohols¹⁴ and of -2.81 kJ/mol for polydisperse ethoxylated alcohols,²⁷ on octyl-silica columns and methanol-water mobile phase. In the case of acetonitrile-water system, the $\Delta\Delta G^\circ$ value is equal to -2.30 kJ/mol, which is lower than that of methanol-water system. All these results are in agreement with the methylene group contribution to the free energy of transfer from water to non-polar stationary phase of hydrophobic compounds in RPC.²⁹

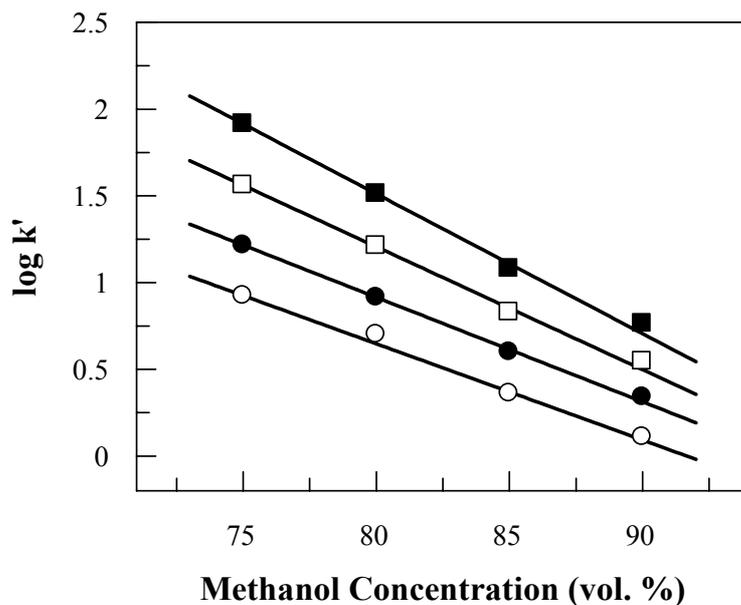


Fig. 1 – The retention factor (k') vs. methanol concentration in water for homogeneously ethoxylated fatty alcohols $C_{10}E_8$ (\circ), $C_{12}E_8$ (\bullet), $C_{14}E_8$ (\square) and $C_{16}E_8$ (\blacksquare), on RP-18 column.

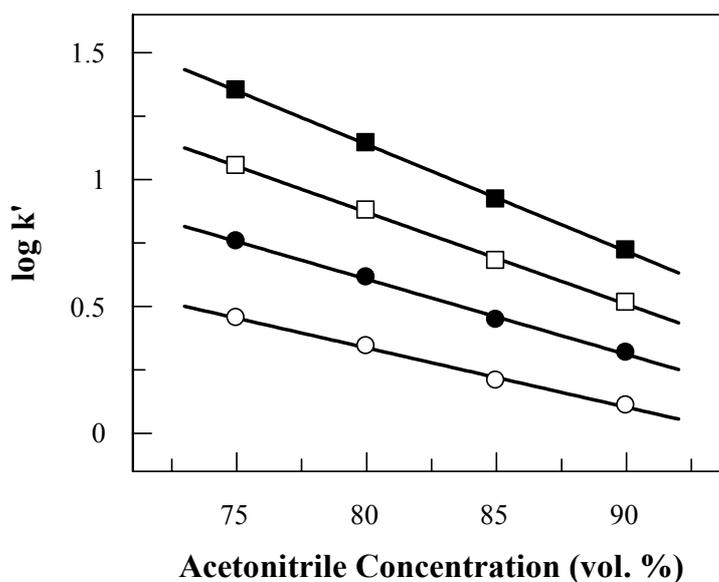


Fig. 2 – The retention factor (k') vs. acetonitrile concentration in water for $C_{10}E_8$ (\circ), $C_{12}E_8$ (\bullet), $C_{14}E_8$ (\square) and $C_{16}E_8$ (\blacksquare), on RP-18 column.

Table 1

The best fit parameters obtained from simulations with eq. (5) of homogeneously ethoxylated fatty alcohols with methanol-water and acetonitrile-water eluents.

Organic modifier	Surfactants	$\log k_w$	$-b \times 10^2$	$s_b \times 10^2$ ^(a)	r ^(b)
Methanol	$C_{10}E_8$	5.09	5.55	0.31	0.9985
	$C_{12}E_8$	5.73	6.02	0.48	0.9968
	$C_{14}E_8$	6.87	7.08	0.82	0.9934
	$C_{16}E_8$	7.96	8.06	1.01	0.9923
Acetonitrile	$C_{10}E_8$	2.21	2.34	0.22	0.9960
	$C_{12}E_8$	2.98	2.97	0.23	0.9971
	$C_{14}E_8$	3.76	3.63	0.20	0.9985
	$C_{16}E_8$	4.51	4.22	0.12	0.9996

^(a) Standard error of fit; ^(b) Correlation coefficient.

Figure 3 presents the retention behavior of homogeneous ethoxylated nonylphenols with 7 and, respectively, 14 EO units in the molecule. The elution was done with methanol-water mixtures. We showed the data for only two surfactants because the retention plots of the NPE_m compounds having different number of ethylene oxide groups in the molecule are linear and very closed to each-other. However, the slopes change gradually with the ethoxylation degree of the surfactants. Moreover, at 90 % (vol.) methanol, the

retention decreases slowly with the EO unit number of the surfactants whereas, at 75 % (vol.) methanol in eluent, a slight increasing of retention with ethoxylation degree is observed. In between, namely at about 20 % (vol.) water in the eluent, the retention has an inversion point. The computed $\log k_w$ values of NPE_m are summarized in Table 2 together with the slopes and the correlation coefficients of fit. The $\log k_w$ values are very closed to each-other, which denotes similar hydrophobic properties of these compounds.

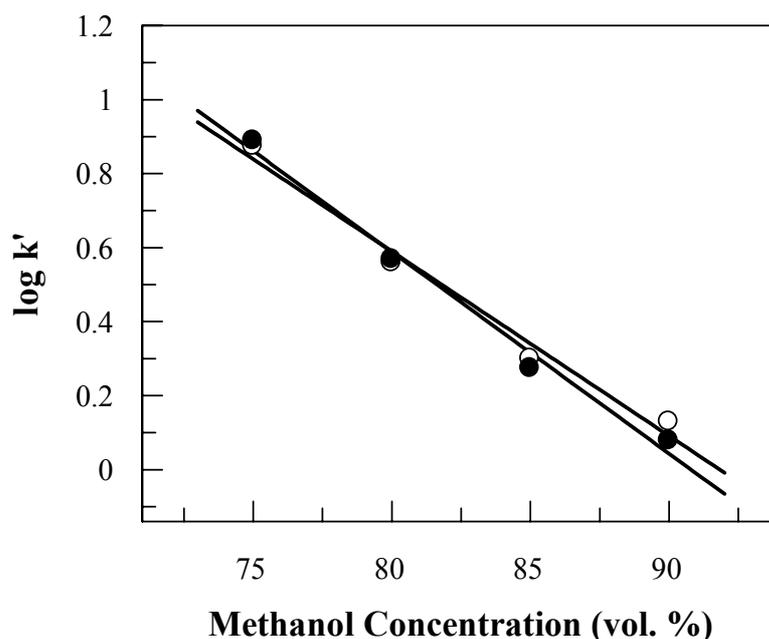


Fig. 3 – The plots of retention factor (k') vs. methanol concentration in water for homogeneous ethoxylated nonylphenols with 7 (NPE₇) (○) and respectively, 14 (NPE₁₄) (●) ethylene oxide units in the molecule.

We observed a small increasing trend of the retention with the EO unit number of the NPE_m. This phenomenon was also noticed for homogeneous and polydisperse ethoxylated nonylphenols on reversed-phase systems.^{13,14} It is rather unusual because k_w is regarded as a standard chromatographic parameter of hydrophobicity. Other reports showed that the linearity of the retention plots in RPLC are not valid for a wide range of mobile phase composition and the extrapolated $\log k_w$ values are different from those experimentally measured.³⁰ To overcome this, we used another parameter, namely the chromatographic hydrophobicity index, ϕ_0 , originally introduced by Valko and Slégel.³¹ The ϕ_0 data summarized in Table 2 reveal that in contrast with k_w , the values of ϕ_0 slightly decrease with the EO number and denote that ϕ_0 is more appropriate to characterize the hydrophobicity of ethoxylates than k_w .

Figure 4 shows the change of $\log k'$ with acetonitrile concentration for NPE_m. One observes a different trend from that recorded when eluting with methanol-water. The retention plot was almost linear only for NPE₇. For surfactants with higher ethoxylation degree, the linearity holds only for water contents in the eluent higher than 20 % (vol.). In the eluent range rich in acetonitrile, the longer the PEO chain, the higher is the deviation from linearity. At 90 % and 85 % (vol.) acetonitrile in the eluent, the retention increases with the EO unit number of the surfactant, which is a reverse situation to that in the methanol-water system. When the acetonitrile content equals 80 % (vol.), the $\log k'$ values become almost the same. At smaller content of acetonitrile the retention of ethoxylates reverses and at 75 % (vol.) acetonitrile it slowly decreases with the PEO chain length.

This behavior is similar to that observed for polydisperse ethoxylated nonylphenols in RPLC.²¹ In that case, the inversion point was at about 80 % (vol.) acetonitrile in the eluent although the retention plots of the surfactants crossed gradually in a range of eluent composition not so narrow as for homogeneous ethoxylates. This difference was related to the distribution of PEO chain length. By

comparing the behavior of NPE_m in methanol-water and acetonitrile-water elution systems (see Figures 3 and 4), one observes a tendency of changing the retention with the water content of the eluent, the effect being very strong in the case of higher ethoxylates.

Table 2

The best fit parameters obtained from simulations with eq. (5) of homogeneously ethoxylated nonylphenols with methanol-water mobile phase

Surfactants	$\log k_w$	$-b \times 10^2$	$s_b \times 10^2$ ^(a)	r ^(b)	ϕ_0
NPE ₇	4.63	5.05	0.42	0.9932	91.68
NPE ₈	4.57	4.98	0.46	0.9916	91.83
NPE ₉	4.62	5.03	0.44	0.9925	91.76
NPE ₁₀	4.64	5.06	0.53	0.9894	91.57
NPE ₁₁	4.72	5.16	0.46	0.9923	91.40
NPE ₁₂	4.82	5.27	0.44	0.9932	91.36
NPE ₁₃	4.86	5.33	0.45	0.9932	91.18
NPE ₁₄	4.91	5.40	0.43	0.9937	90.95
NPE ₁₅	4.95	5.45	0.41	0.9944	90.79

^(a) Standard error of fit; ^(b) Correlation coefficient.

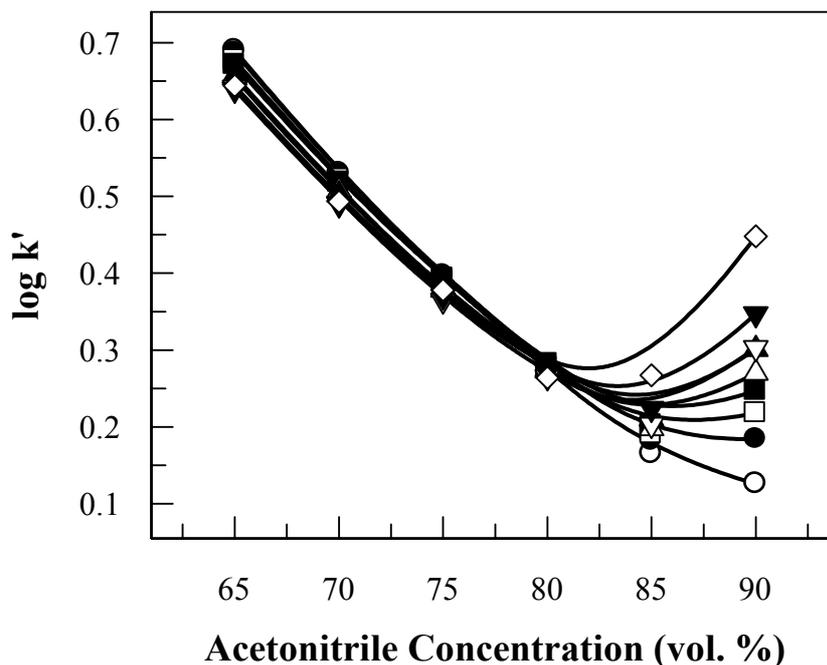


Fig. 4 – The dependence of retention factor (k') on the acetonitrile concentration in water, for NPE₇ (○), NPE₈ (●), NPE₉ (□), NPE₁₀ (■), NPE₁₁ (△), NPE₁₂ (▽), NPE₁₃ (▲), NPE₁₄ (▼) and NPE₁₅ (◇), on the RP-18 column.

The results obtained by us for ethoxylated nonionic surfactants in RPLC show that retention is related to the eluent, a phenomenon that is well described by the solvophobic theory^{29,32} or its simplified form.³³ According to these theories, the retention is a reversible hydrophobic association of

the elute with the solvated hydrocarbonaceous chains bonded to the stationary phase. This effect manifests itself as the tendency of the non-polar or weakly polar elute to reduce its surface area exposed to water. The magnitude of retention depends on the energy balance of the elute and of

eluent with the stationary phase, and on the interactions between elute and the eluent. The analysis of the retention data shows that the last two interactions and the solvation, in which the effect of the mobile phase is involved, play a dominant role in the chromatographic process. Previously, we put forward a relationship between the retention behavior of polydisperse ethoxylated nonylphenols in RPLC and the surface tension and the viscosity of the eluent.²¹ Although the surface tension alone was not able to explain the surfactant retention, the corroboration with viscosity data indicated a weaker interaction between acetonitrile and water than between methanol and water. Therefore, we decided to investigate other parameters characterizing the eluent such as the molar refraction, R , and the dielectric constant, ϵ , and to correlate them with surfactant retention.

It is known that the molar refraction is a measure of electronic polarization of the molecules. The excess molar refraction (R^E) indicates changes in the electrical properties of the

solution by alteration of its composition.^{22,23} The dependence of R^E on the composition of methanol-water and acetonitrile-water mixtures is shown in Figure 5. The R^E values are negative for both mixtures, which proves a deviation from the ideality and a modification of polar interactions between the organic solvent and the water molecules. One may observe that in the region rich in organic solvent, R^E decreases linearly for both eluents when the water content increases. The phenomenon lasts up to a water content of about 35 % (vol.). In the case of acetonitrile-water mixture, R^E is more negative indicating a stronger change in the polarity of the mixture. This distinction seems to indicate different structural modifications appearing in the eluent mixtures by changing their composition. However, the comportment of R^E in this range does not offer satisfactory information about the structural modifications appearing in the eluents, which could be related to the unusual behavior of surfactants.

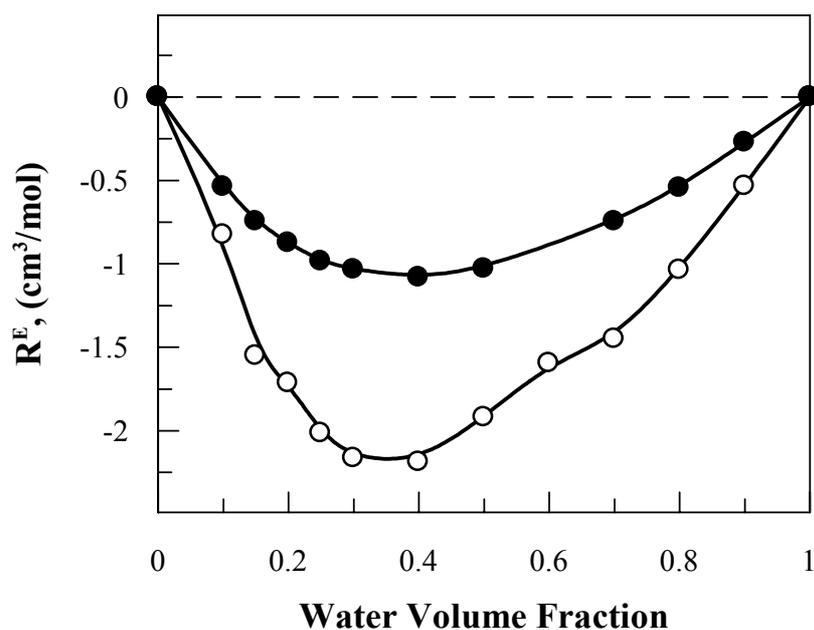


Fig. 5 – The change of the excess molar refraction (R^E) with the hydro-organic mixture composition, at 40°C: acetonitrile (○), methanol (●).

Figure 6 illustrates the deviation of the excess dielectric constant, ϵ^E , from ideality for the eluent mixtures used by us. In the case of methanol-water, the ϵ^E values are negative in the range of 0 to 80 % (vol.) water and positive for higher water concentrations. For methanol-rich solutions, the ϵ^E values decrease with the water content up to about 40 % (vol.). Then, ϵ^E increases and reaches a maximum at 90 % (vol.) water. The results are in

good accord with the previously published data indicating changes in the structure of the methanol-water clusters.^{34,35} For methanol molar fractions, x_M , smaller than 0.3, the mixture consist of tetrahedral-like water clusters, at $0.3 < x_M < 0.7$, the water cluster are gradually replaced by methanol clusters without significant disruption of the water lattice and for $x_M > 0.7$, the chain clusters of methanol become predominant.³⁵

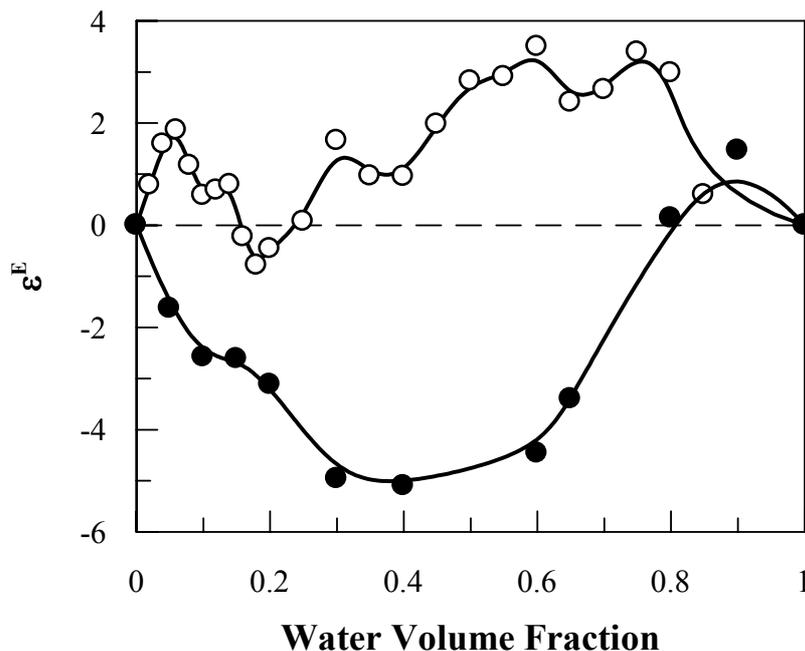


Fig. 6 – The excess dielectric constant (ϵ^E) as a function of water volume fraction, at 40°C: acetonitrile (○), methanol (●).

As regards the acetonitrile-water mixtures, the change of ϵ^E against the water volume fraction forms two regions of positive values and one of negative ones, which indicate several structural regions. Although acetonitrile is completely water-miscible, various physical and chemical techniques like the excess volume, viscosity, dielectric constant and acid-base properties revealed three main structurally different regions.^{25,36} The data presented in Figure 6 also suggest the existence of such structural regions. In the acetonitrile-rich region there is a maximum of ϵ^E at about 8 % (vol.) water, and one may reasonably assume that the original structure of acetonitrile is progressively disturbed by addition of water. At higher water content, ϵ^E becomes lower, and attains a minimum at about 20 % (vol.) water. This point is probably the limit of that range of compositions called "microheterogeneity region" in which aggregates of acetonitrile molecules coexist with the aqueous lattice. The nature of solution structure in the microheterogeneity region has not exactly been established, but some arguments support the supposition that in the water microclusters the H-bonded structure is enhanced relative to that of pure water.³⁷ In the water-rich range, namely at water contents above 80 % (vol.), we observed a decrease of ϵ^E which allows to assume the raising of the aqueous framework with acetonitrile filling the cavities inside the water structure.

Although the methanol-water and acetonitrile-water eluents are generally thought binary mixtures on the macroscopic level, on the microscopic scale they are ternary mixtures consisting of organic solvent molecules, organic solvent-water associated molecules, and water molecules. The ternary nature of the aqueous mixtures must be taken into account in attempting to explain the retention of elute in reversed-phase liquid chromatography. It is known that the methanol and acetonitrile differ in the solvent strength for alkyl chains and in their hydrogen-bonding ability.³⁸ Thus, methanol is able to form hydrogen bonds by accepting or donating protons, whereas acetonitrile is unable to form them to any appreciable extent. Consequently, in the region rich in organic solvent, the chain-like clusters of methanol including water form a network through hydrogen bonding between different chains. In the acetonitrile-water mixtures, individual water molecules interact with individual acetonitrile molecules with little disruption of the weak dipole-dipole framework of the main component. Unlike acetonitrile, the hydrogen-bonding solvents such as water and methanol solvate very slowly the non-polar alkyl chains.

The organic solvent and the mobile phase composition affect the character of the stationary phase. At high modifier concentration, the solvent-chain interactions overcome the intra- and intermolecular interactions of the alkyl chains and their structure becomes brush-like. It gives a larger

carbon surface area to the solvent molecules and confers to the elutes a stationary phase more “non-polar” than the mobile phase.³⁸ Due to these, in the reversed-phase liquid chromatography the stationary phase is considered as a ternary combination of bonded organic moiety, adsorbed solvent molecules and residual silanols on the silica surface, but its formation is a dynamic process controlled by the mobile phase. During solvation, the bonded alkyl layer can be selectively enriched in organic modifier through dispersion interaction and the amount of modifier depends on its strength for alkyl chains and the concentration in the mobile phase. Some reports showed that the compositions of both stationary and mobile phases are similar for methanol as organic modifier, but the stationary phase contains an excess of organic solvent in comparison to its amount in the mobile phase for acetonitrile even at low acetonitrile concentrations.³⁹

The organic modifier extracted by the stationary phase as a consequence of hydrophobic expulsion from the aqueous mobile phase also interacts with the elute molecule. This makes the RPLC retention process much more complex than the simple interaction of elute with the bonded alkyl chains. In the ethoxylated surfactant series investigated by us the members have various non-polar alkyl and polar PEO chain length and their overall hydrophilic-lipophilic properties are different. As a consequence, the interactions between the surfactants and the components of the mobile and stationary phase change as well. Taking into account the amphiphilicity of ethoxylated surfactants one may admit that the hydrophobic alkyls can penetrate in-between the bonded non-polar octadecyl chains of the fur-like stationary phase whereas the hydrophilic PEO chains are oriented towards the bulk polar mobile phase. The very closed retention values of $C_{10}E_8$ and NPE_m with methanol-water (see Tables 1 and 2), denote comparable hydrophobic character of these ethoxylates. The linear increase of retention with the alkyl carbon number of the C_nE_8 for both eluting systems proves the dominant role of hydrophobic interactions and suggests a retention mechanism involving a deeper insert of longer alkyl of surfactants between the chains bonded to the stationary phase. However, the retention values of C_nE_8 in acetonitrile-water are lower than in methanol-water, which denotes a higher solvent strength of acetonitrile in comparison with methanol, and a better solvation of the hydrophobic segments of elute molecule.

As shown in Figures 3 and 4, the retention behavior of NPE_m in methanol-water is different from that in acetonitrile-water. Although in both systems, the retention of ethoxymers inverses at 80 % (vol.) organic solvent in the eluent, the retention order and the linearity of the plots are different for methanol and acetonitrile. For elutes differing only by the EO group number, we deduced some irregularities in the interaction between the hydrophilic PEO chain and the species of the elution systems. These irregularities were previously explained by using the two-state conformation of PEO chain.⁹ According to this theory, the extended “zigzag” and the compact “meander” conformers, have different retention properties, which are influenced by the number of EO units, the nature and composition of hydro-organic eluent, and temperature. On the other hand, the retention of ethoxylates is associated to effects of solvation and polarity, which depend on the water content in the eluent. As the present molar refraction and dielectric data show, the increase of water content in the hydro-organic mixtures entails changes in eluent polarity and in solvation of PEO chain and consequently, the retention of ethoxymers gradually changes. The number of EO groups favors the solvation of PEO chain with methanol and water molecules by hydrogen bonds and this could explain the decrease of surfactant retention as a function of their ethoxylation degree at 90 % (vol.) methanol. With acetonitrile such interactions are not possible, and at the same mobile phase composition the retention increases with the EO group number. Such an irregular behavior in reversed-phase chromatography was also noticed for some crown ethers containing EO groups in their molecule.⁴⁰ It has been explained as being the result of both solvophobic (hydrophobic) effect and silanophilic interactions; the latter occurring between the oxyethylene chain and the residual silanol groups, provided no specific solvent effects or conformation changes are involved. The conformational modifications of the alkyl-bonded phase towards the stretched form within the acetonitrile-water eluent allow the access of polar EO groups to the residual silanols of the stationary phase.

The irregular influence of the mobile phase composition on the behavior of polyethoxylates with acetonitrile as organic modifier was also thermodynamically evaluated.¹⁶ The changes in the thermodynamic parameters depend on the solvation of PEO chains by the mobile phase, their conformational modification, the interactions with silanols, or effects associated to these processes.

All of them are affected by the acetonitrile-water ratio. Thus, the modification of solvation induces the change of conformation and the conformers have different stationary-mobile-phase distribution. Recently, the unusual temperature dependence of ethoxylated fatty alcohols in RPLC was attributed to the opposite contribution of alkyl and PEO chains to the retention.¹⁷ The retention was enthalpically favorable for alkyl, unfavorable for PEO, entropically favorable for PEO and unfavorable for alkyl, and allowed explaining the inversion point of ethoxymer retention.

CONCLUSIONS

In the investigated reverse-phase chromatographic systems, the retention of ethoxylated fatty alcohols ($C_{10-16}E_m$) increases with the alkyl chain length of the surfactants and with the water concentration in the eluent. The effect of the ethoxylation degree on the retention of ethoxylated nonylphenols (NPE₇₋₁₅) is influenced by the nature of the organic modifier (methanol or acetonitrile) and by the eluent composition. To explain these, the changes of excess molar refraction (R^E) and of excess dielectric constant (ϵ^E) as a function of eluents composition were considered. The differences in the behavior of these parameters indicate structural changes of the hydro-organic mixtures, which affect the interactions between the ethoxylates and the mobile and the solvated stationary phase. The hydrophobic interactions, the associated solvation and polarity effects, the concurrent contribution of the alkyl and PEO chains to the chromatographic process as well as the silanophilic interaction between the PEO units and the accessible residual silanols were used to explain the behavior of ethoxylated surfactants.

REFERENCES

- J. B. Jeong, J. Y. Kim, J. H. Cha and J. D. Kim, *Colloids Surf. A*, **2002**, *207*, 161-167.
- H. Kunieda and M. Yamagata, *Langmuir*, **1993**, *9*, 3345-3351.
- J. L. Salager, N. Marquez, A. Graciaa and J. Lachaise, *Langmuir*, **2000**, *16*, 5534-5539.
- J. J. Morelli and G. Szajer, *J. Surfact. Deterg.*, **2001**, *4*, 75-83.
- M. C. Allen and D. E. Linder, *J. Am. Oil Chem. Soc.*, **1981**, *58*, 950-957.
- P. Jandera, J. Urbánek, B. Prokeš and J. Churaček, *J. Chromatogr.*, **1990**, *504*, 297-318.
- D. F. Anghel, M. Balcan, A. Voicu and M. Elian, *J. Chromatogr. A*, **1994**, *668*, 375-383.
- P. Jandera, M. Holcapek and G. Theodoridis, *J. Chromatogr. A*, **1998**, *813*, 299-311.
- W. R. Melander, A. Nahum and C. Horvath, *J. Chromatogr.*, **1979**, *185*, 129-152.
- Z. Wang and M. Fingas, *J. Chromatogr.*, **1993**, *673*, 145-156.
- T. Kamiyusuki, T. Monde, F. Nemoto, T. Konakahara and Y. Takahashi, *J. Chromatogr. A*, **1999**, *852*, 475-485.
- P. Chaimbault, C. Elfakir and M. Lafosse, *J. Chromatogr. A*, **1998**, *797*, 83-91.
- M. Balcan, D. F. Anghel and C. Bobică, *Rev. Roum. Chim.*, **1998**, *43*, 1105-1112.
- M. Balcan, D. F. Anghel, A. Voicu and N. Cornilescu, *Rev. Roum. Chim.*, **1999**, *44*, 369-375.
- C. H. Lochmüller, M. A. Moebus, Q. Liu, C. Jiaang and M. Elomaa, *J. Chromatogr. Sci.*, **1996**, *34*, 69-76.
- K. Lemr, J. Ševčík and J. Hlaváč, *J. Chromatogr. A*, **2003**, *1021*, 19-24.
- D. Cho, S. Park, J. Hong and T. Chang, *J. Chromatogr. A*, **2003**, *986*, 191-198; 199-206.
- M. Balcan, D.F. Anghel, A. Voicu and D.C. Balcan, *Colloids Surf. A*, **2002**, *204*, 141-151.
- M. Balcan and D. F. Anghel, *Colloids Surf. A*, **2003**, *221*, 1-8.
- M. Balcan and D. F. Anghel, *Ann. West Univ. Timisoara*, **2003**, *12*, 837-846.
- M. Balcan, D. F. Anghel, D. Donescu and A. N. Galatanu, *Rev. Roum. Chim.*, **2006**, *51*, 299-306.
- C. Yanes, A. Maestre, P. Pérez-Tejeda and J. J. Calvente, *J. Chem. Eng. Data*, **1993**, *38*, 512-515.
- T. M. Aminabhavi, M. I. Aralaguppi, S. B. Harogoppad and R. H. Balundgi, *J. Chem. Eng. Data*, **1993**, *38*, 31-39.
- V. Raicu, *Meas. Sci. Technol.*, **1995**, *6*, 410-414.
- C. Moreau and G. Douhét, *J. Chem. Thermodyn.*, **1976**, *8*, 403-412.
- L. R. Snyder, J. W. Dolan and J.R. Gant, *J. Chromatogr.*, **1979**, *165*, 3-30.
- M. Balcan and D. F. Anghel, *Rev. Roum. Chim.*, **1998**, *43*, 1043-1047.
- L. R. Snyder, J. J. Kirkland, "Introduction to Modern Liquid Chromatography", John Wiley & Sons, Inc., New York, 1979, p. 257.
- A. Vailaya and C. Horvath, *J. Chromatogr. A*, **1998**, *829*, 1-27.
- M. M. Hsieh and J. Dorsey, *J. Chromatogr.*, **1993**, *631*, 63-78.
- K. Valko and P. Slégel, *J. Chromatogr.*, **1993**, *631*, 49-61.
- C. Horvath, W. Melander and I. Molnar, *J. Chromatogr.*, **1976**, *125*, 129-156.
- A. Vailaya and C. Horvath, *J. Phys Chem B*, **1997**, *101*, 5875-5888.
- S. Mashimo, T. Umehara and H. Redlin, *J. Chem. Phys.*, **1991**, *95*, 6257-6260.
- T. Takamuku, T. Yamaguchi, M. Asato and M. Matsumoto, *J. Phys. Sci. Sect A-A*, **2000**, *55a*, 513-525.
- J. Catalan, C. Diaz and F. Garcia-Blanco, *Org. Biomol. Chem.*, **2003**, *1*, 575-580.
- Y. Marcus and Y. Migron, *J. Phys. Chem.*, **1991**, *95*, 400-406.
- C. R. Yonker, T. A. Zwer and M. F. Burke, *J. Chromatogr.*, **1982**, *241*, 269-280.
- R. M. McCormick and B. L. Korger, *Anal. Chem.*, **1980**, *52*, 2249-2257.
- K. E. Bij, Horvath C. W. Melander and A. Nahum, *J. Chromatogr.*, **1981**, *203*, 65-84.