

*Dedicated to Professor Dr. ALEXANDRU T. BALABAN, member of the Roumanian Academy on the occasion of his 75th anniversary*

## EFFECT OF THE MOBILE PHASE COMPOSITION ON THE RETENTION BEHAVIOR OF POLYDISPERSE ETHOXYLATED NONYLPHENOLS IN REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Marieta BALCAN,<sup>\*a</sup> Dan F. ANGHEL,<sup>a</sup> Dan DONESCU<sup>b</sup> and Anca-Nicoleta GALATANU<sup>c</sup>

<sup>a</sup>Department of Colloids, “I. G. Murgulescu” Institute of Physical Chemistry, Spl. Independenței 202, Post Office 12, P. O. Box 194, 060021 Bucharest, Roumania

<sup>b</sup>Researcher Center for Polymer and Plastic Materials, Spl. Independenței 202, 060021 Bucharest, Roumania

<sup>c</sup>UMR 7099/Institute of Biological Physical Chemistry – C.N.R.S., 13 rue Pierre-et-Marie-Curie F-75005 Paris, France

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The paper presents the effect of organic modifiers on the reversed-phase retention behavior of the polydisperse ethoxylated nonylphenols (NPEO<sub>7-15</sub>). The retention plots (log  $k'$  vs. organic solvent concentration) obtained with methanol-water as eluent are linear, while those with acetonitrile-water are not and the deviation from linearity increases with the ethoxylation degree of the surfactant. For both elution systems the retention plots reverse at about 20% (vol.) water in the eluent. The physical properties of the hydro-organic eluents such as surface tension and viscosity were investigated as a function of the binary mixture composition and related to the retention data of the surfactants. The surface tension values decreased linearly with the methanol content in the eluent, but remained constant in the case of acetonitrile-water mixtures. The viscosity data indicated that acetonitrile interacts with water more feebly than methanol does. This suggests that acetonitrile molecules unassociated with water in the mobile phase adsorb on the hydrophobic stationary phase. Between the ethylene oxide (EO) groups close to the alkyl chains of the surfactants and the acetonitrile layer adsorbed onto the stationary phase specific interactions appear, which account for the increase of the surfactant retention with the length of the poly(ethylene oxide) (PEO) chain. Considerations about the retention mechanism of these compounds in reversed-phase systems are put forward.

### INTRODUCTION

Nonionic surfactants such as ethoxylated nonylphenols are amphiphilic compounds obtained by condensation of ethylene oxide (EO) with alkylphenols. Commercial products are never pure materials, being complex mixtures of oligomers with different numbers of EO units and having isomerism in the alkyl chain and in the position of substitution to the aromatic ring. The interest in ethoxylated surfactants is due to their broad use in many industrial and household applications as detergents, emulsifiers, cosmetic additives, agents for enhanced oil recovery, nanoparticle and mesoporous material synthesis, etc.<sup>1-3</sup> Their properties and applications greatly depend upon the equilibrium between the hydrophobic tail and the polar poly(ethylene oxide) (PEO) chain.

A frequently used technique to characterize the ethoxylated nonionic surfactants is the high-performance liquid chromatography (HPLC).<sup>4,5</sup> Generally, the normal-phase liquid chromatography (NPLC) with bare silica or chemically bonded diol, nitrile or amino phases separates the ethylene oxide oligomers,<sup>6-9</sup> whereas the reversed-phase liquid chromatography (RPLC) with alkyl bonded phases gives information about the hydrophobic tail.<sup>9-11</sup> However, it is worth mentioning in this context, the separation of ethoxylates according to the ethylene oxide units achieved by the suitable RPLC combination of the stationary and mobile phase<sup>12-14</sup> or by using hyphenated exclusion-adsorption chromatography.<sup>15-17</sup>

\* Corresponding author: mbalcan@icf.ro

An issue of debate in RPLC with alkyl bonded stationary phases and hydro-organic eluents is the retention process of the elute. The major problem arising is whether the molecules are retained by an adsorption or by a partition mechanism. There are two attempts namely, the solvophobic<sup>18</sup> and the partition theory,<sup>19,20</sup> which try to explain the driving force and the fundamental retention mechanism. Nonetheless, even the improved solvophobic theory with isolated solvated hydrocarbon chains does not distinguish between the adsorption or partition mechanism.<sup>21</sup> Depending on the mobile phase composition, the bonded chains can unfold in organic modifier rich eluents or collapse in those rich in water. Irrespective of stationary phase configurations, the retention in RPLC is governed by the hydrophobic interactions and depends on the magnitude of the contact area formed upon association of the elute molecule with the isolated solvated ligands. The difficulty in elucidating the mechanism of retention lies in numerous interactions that the elute may undergo in both the stationary and the mobile phases.

At the same time, there are a few reports connecting the retention characteristics of ethoxylated surfactants in RPLC with their physico-chemical properties. Some studies deal with the effect induced by the hydrophobic and hydrophilic moieties of the surfactants on their RPLC retention.<sup>22-25</sup> They reveal a stronger contribution of the hydrophobic part than of the hydrophilic PEO moiety. Good correlations were established between the retention data and the hydrophilic-lipophilic properties of the surfactants.<sup>22,24</sup> The data on the retention mechanism of these surfactants in RPLC showed that the retention process is quite complex and depends on the support, the organic modifier and its concentration in the mobile phase.<sup>26-28</sup>

Our previous work on the behavior of ethoxylated nonionic surfactants in reversed-phase systems with octyl- and octadecyl-silica columns and methanol-water eluents revealed a good relationship between the retention data and the hydrophobicity parameters such as the hydrophile-lipophile balance (HLB) and the oil/water partition coefficient ( $K_{ow}$ ).<sup>29,30</sup> The thermodynamic investigation demonstrated that irrespective of the eluent composition and the length of the alkyl chain bonded to the stationary phase, the ethoxylated surfactants are retained through a similar mechanism.<sup>31-33</sup>

In the present study, we considered the role of the organic modifier on the retention behavior of polydispersed ethoxylated nonylphenols in RPLC. The surfactants investigated had the same hydrophobic part but different average ethoxylation degrees. The chromatographic data obtained with the acetonitrile-water eluent were compared with those with methanol-water. To shed light on the molecular interactions involved in the retention, the physical properties of the eluents such as the surface tension and viscosity were determined, and their dependence on composition was correlated with the changes observed in the retention of ethoxylates. The mechanism of surfactant retention was also discussed.

## EXPERIMENTAL

### Chemicals

The nonionic surfactants used were samples of polydisperse ethoxylated nonylphenols (NPEO<sub>m</sub>) purchased from Petrochemical Plant Brazi, Roumania (NPEO<sub>7</sub>), BASF AG, Ludwigshafen, Germany (NPEO<sub>10</sub>), Dow Chemical Inc., Wilmington, DE, USA (NPEO<sub>15</sub> and NPEO<sub>30</sub>) and Polymer Group, Wilton, Middlesbrough, UK (NPEO<sub>40</sub>). Methanol and acetonitrile were of HPLC-grade reagents. Doubly distilled water having an electrical conductivity lower than 1.5  $\mu$ S/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 on a Hewlett-Packard (Boeblingen-Germany) model 1084 B, liquid chromatograph, fitted with the variable wavelength UV detector model 79 875 A, set at 280 nm. The analytical column (200  $\times$  4.6 mm I.D.) packed with irregular octadecyl-silica (RP-18, 10  $\mu$ m) was purchased from Hewlett-Packard (Waldbronn, Germany). The mobile phase consisted of hydro-organic mixtures having different volume fractions of methanol and acetonitrile. The flow rate was of 1 mL/min. The running solutions for HPLC had a concentration of 10 mg/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  $\mu$ L.

Each surfactant sample was eluted in the isocratic mode. The result was a symmetric peak indicating that the reversed-phase chromatographic systems used are not suitable to separate the surfactants according to the number of ethylene oxide groups in the molecule. The retention values were reported in terms of the capacity factor,  $k'$ , calculated according to  $k' = (t_r - t_0)/t_0$ . The retention time of the surfactant,  $t_r$ , was the mean of at least three reproducible determinations and the dead time,  $t_0$ , was measured for each eluent with deuterium oxide. The average ethoxylation degrees of the surfactants were determined by normal-phase HPLC as described elsewhere.<sup>7,9</sup>

### Surface tension

The surface tension,  $\gamma$ , as a measure of the uncompensated surface energy of the hydro-organic solution was measured at 40°C by the ring method.<sup>34</sup>

### Viscosity

The viscosity measurements were carried out with an Ostwald viscometer having a flow time of 300 sec for water at 40 °C. The relative viscosity of the solutions was determined as:

$$\eta_{\text{rel}} = t / t_w \quad (1)$$

where,  $t$  is the solution flow time and  $t_w$  is the flow time of water. The dynamic viscosity (cP) was calculated by multiplying the relative viscosity with the solution density,  $\rho$ , according to equation (2):

$$\eta = \rho \cdot \eta_{\text{rel}} \quad (2)$$

The solution density was determined with the aid of pycnometer.

The viscosity deviation  $\Delta\eta$  was calculated from the viscosity values of binary mixtures with the aid of equation (3):<sup>35</sup>

$$\Delta\eta = \eta_m - \phi\eta_1 - (1 - \phi)\eta_2 \quad (3)$$

where, subscripts 1, 2 and m refer to water, organic solvent, and respectively to their mixture, and  $\phi$  is the volume fraction of water.

## RESULTS AND DISCUSSION

Figs. 1 and 2 illustrate the semilog plots of the capacity factor of polydisperse ethoxylated nonylphenols as a function of methanol and, respectively, acetonitrile concentration in the hydro-organic eluent. In both systems, there is an apparent trend of increasing retention with the water content of the eluent, except for the higher ethoxylated surfactants on the eluent range rich in acetonitrile.

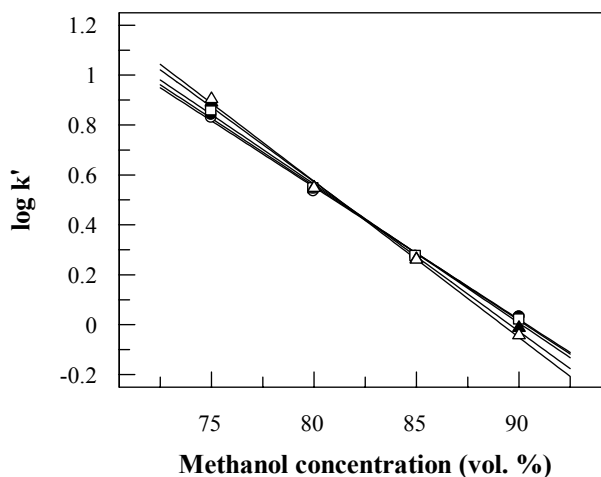


Fig. 1 – The variation of  $\log k'$  with the methanol content in the mobile phase for NPEO<sub>7</sub> (○), NPEO<sub>10</sub> (●), NPEO<sub>15</sub> (□), NPEO<sub>30</sub> (▲) and NPEO<sub>40</sub> (△) on the RP-18 column.

Using the methanol-water eluent, the  $\log k'$  vs.  $\phi$  plots are linear and very close to each other on the entire range of eluent composition (see Fig. 1). In RPLC, the linearity of the retention plots is common to the hydrophobic compounds,<sup>37</sup> which agrees with the solvophobic theory.<sup>18</sup> The slopes of the plots are very close to each other and account for the similar retention properties of the surfactants. However, they slightly depend on the average ethoxylation degree of the surfactants, and one may notice a small effect of the PEO chain length on retention. This determined us to investigate the variation of retention with the ethoxylation degree of the surfactants at various eluent compositions. With the mobile phase rich in methanol, namely at 90% (vol.) (see Fig. 1), the retention decreases barely with the EO group number, which means that the interaction between the surfactants and the mobile phase strengthens with the PEO chain length of the surfactant. By increasing the water content in the mobile phase, the retention times increase, become closer

to each other, and at about 82% (vol.) methanol are the same. When the water content reaches 25% (vol.), the retention has a small increase with the EO unit number. This means that the interaction between the surfactant and the stationary phase intensifies with the length of the PEO chain. The inversion point of retention at about 82% (vol.) methanol in eluent is in accord with that previously observed for this class of surfactants in similar chromatographic systems.<sup>12,29,30</sup>

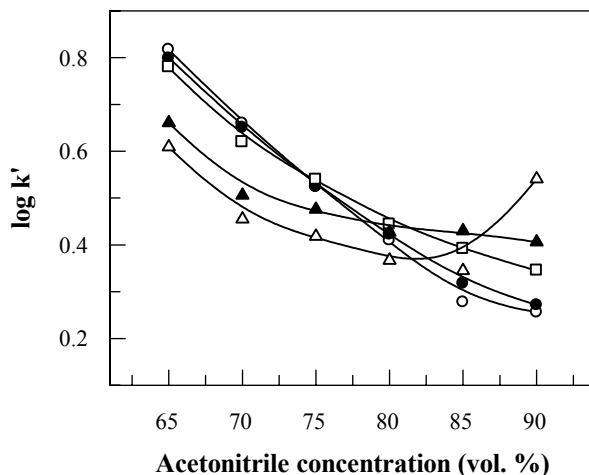


Fig. 2 – The variation of  $\log k'$  with the acetonitrile concentration in the eluent for NPEO<sub>7</sub> (○), NPEO<sub>10</sub> (●), NPEO<sub>15</sub> (□), NPEO<sub>30</sub> (▲) and NPEO<sub>40</sub> (△) on the RP-18 column.

The data in Fig. 2 show the change of the capacity factor of the surfactants when eluted with acetonitrile-water. There are differences but also similarities of these data with those obtained by using methanol as organic modifier. With the acetonitrile-water eluent, the linearity of retention plots does not hold for all the surfactants and the deviation from linearity increases with the ethoxylation degree. At 90% (vol.) acetonitrile in the eluent, the retention values increase obviously with the length of the PEO chain. This situation is different from that in the methanol-water system and shows that the interaction between the stationary phase and the surfactant becomes stronger by increasing the PEO chain length. When the acetonitrile content in the mobile phase decreases, the retention values of the surfactants get closer to each other. The plots of NPEO<sub>7</sub>, NPEO<sub>10</sub> and NPEO<sub>15</sub> cross at about 73% (vol.) acetonitrile, whereas the cross point for NPEO<sub>30</sub> and NPEO<sub>40</sub> is at 86% (vol.) acetonitrile. At about 80% (vol.) acetonitrile the retention data of all investigated surfactants seem to be the closest to each other. One may infer that with methanol-water and acetonitrile-water eluents, the surfactant retention reversed at about 20% (vol.) of water in the eluent. However, the retention order of the surfactants in the two systems is contrary. In addition, the effect of a small amount of water on the surfactant retention is more conspicuous when acetonitrile is used as organic modifier.

Previously, an irregular behavior was observed when oligo(ethylene glycol) homologous and their derivatives were separated on RPLC systems.<sup>12</sup> The inversion of the elution order with the mobile phase composition was explained by the two-state conformation of the PEO chain. According to this, the elute has an extended “zigzag” or a compact “meander” conformation, which has different retentions. The equilibrium constant for the conformational transition is determined not only by the number of the EO groups in the surfactant molecule, but also by the organic solvent and its concentration in the aqueous eluent and by temperature.

Within the solvophobic theory of reversed-phase liquid chromatography, the capacity factor is a measure of the relative hydrophobicity.<sup>18</sup> This means that the retention behavior of the elutes is a function of their hydrophobic volume and polarity, and depends on the nature of the organic modifier as well. The chromatographic process for a given elute in different aqueous-organic eluents involves the increase of retention with increasing the carbon number of elute, and it correlates with the eluent physico-chemical properties. Accordingly, we studied the modification of the surface tension and viscosity of the hydro-organic eluents with composition.

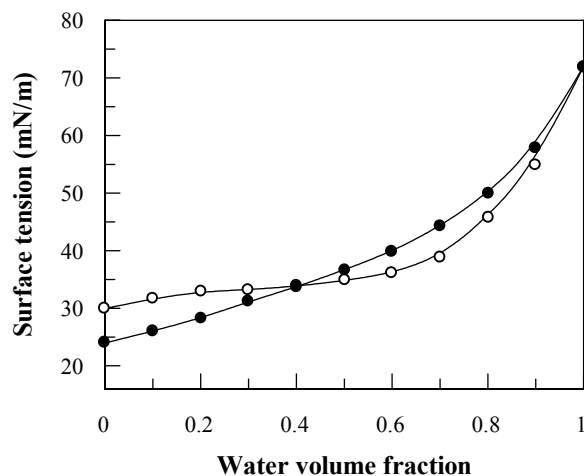


Fig. 3 – Surface tension ( $\gamma$ ) as a function of the water volume fraction of the hydro-organic eluent, at 40°C: acetonitrile (○), methanol (●).

The surface tension of methanol-water and acetonitrile-water mixtures, as a function of water volume fraction is illustrated in Fig. 3. One may observe that the surface tension values of the eluents rich in water are higher than those of the eluents rich in organic solvent. This means that the structure of pure water is strongly disturbed and the free energy of its surface layer changes by adding the organic solvent. This modification occurs almost gradually by increasing the methanol content, which denotes a progressive change of the interactions between water and alcohol molecules. In the case of acetonitrile, the decrease of  $\gamma$  is quite sharp until a volume fraction of about 0.7, thereafter  $\gamma$  remains almost constant. For eluents rich in organic modifier, the surface tensions are slightly higher for acetonitrile than for methanol but the situation reverses above 40% (vol.) water in the eluent. The data of methanol-water and acetonitrile-water mixtures show a crossing point indicating the same  $\gamma$  value for both eluents, which agrees with the data reported in the literature.<sup>18,37</sup>

In the composition range of the hydro-organic eluents used by us, the surface tension changed linearly for methanol, and was unchanged for acetonitrile. In the methanol range from 90 to 75% (vol.), the plots of  $\log k'$  vs. eluent composition are very close to each other and depend slightly on the PEO chain length of the surfactant (see Fig. 1). For acetonitrile in the concentration range from 90 to 65% (vol.), the retention plots of ethoxylates are not linear. The deviation from linearity appears on the eluent rich in acetonitrile side and increases with the ethoxylation degree of the surfactant (see Fig. 2). If only the surface tension is responsible for the behavior of retention, one may expect that below 40% (vol.) water in the eluent the surfactant retention would be smaller in methanol-water than in acetonitrile-water. In fact, this retention order holds for 10% and 15% (vol.) water in the eluent. At 20% (vol.) water, the retention values are almost equal whereas above 20% (vol.) water, they are higher for methanol than for acetonitrile. Compared with the crossing point of the surface tension curves, the inversion point of the retention plots is shifted toward more concentrated organic solvent levels. This corresponds to the previously called “isoelectrope” mixture that gives identical retention times for all eluents independent of their chemical nature.<sup>38</sup> In our opinion, the observed retention of the ethoxylates cannot be explained solely by the effect of surface tension. Previously it was found out that for eluents having  $\pi$  electrons such as ethoxylated nonylphenols, the retention plots do not correlate with the evolution of the surface tension.<sup>36,38</sup> Taking into account the influence of the ethoxylation degree on surfactant retention, one may assume that some special interactions arise between the PEO chains and the mobile and solvated stationary phases.

It is well documented that in liquid chromatography, the hydrodynamic properties of the mobile phase are related to the eluent viscosity and affect the retention of eluite.<sup>39</sup> Therefore, we determined the viscosity for the investigated eluent mixtures and the results are plotted in Fig. 4. The viscosity deviation from ideality ( $\Delta\eta$ ), which is informative on the specific interactions between water and the organic solvent, is different for the two eluents. In the case of methanol-water mixture, the  $\Delta\eta$  values are positive over the whole range of composition. Former dielectric, mass spectrometry and X-ray diffraction measurements on methanol-water mixtures, put into evidence inflection points at water mole fraction,  $x_w$ , of 0.3 and 0.7.<sup>40,41</sup> They were

associated with the following structures: chain clusters of methanol molecules at  $x_w < 0.3$ , clusters of methanol molecules gradually replaced by water in the  $0.3 < x_w < 0.7$  range, and tetrahedral-like water clusters at  $x_w > 0.7$ . Although our viscosity data do not have inflection points, one may assign the positive values of  $\Delta\eta$  to the networking between the methanol and water molecules by hydrogen bondings.

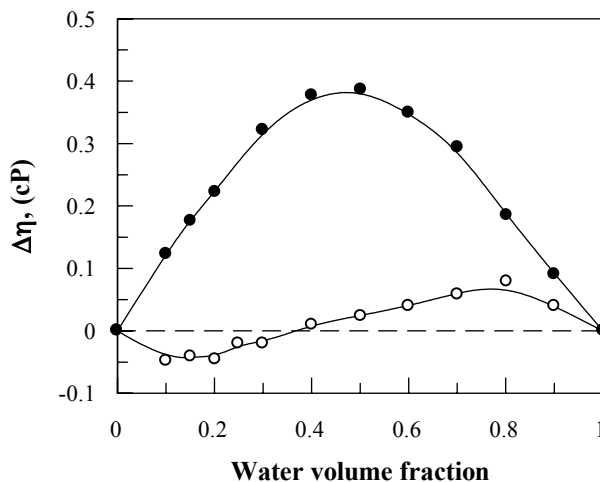


Fig. 4 – Dependence of the viscosity deviation ( $\Delta\eta$ ) on the hydro-organic mixture composition, at 40°C: acetonitrile (○), methanol (●).

Concerning the acetonitrile-water solutions, one may observe that the viscosity has positive deviations from ideality in the water-rich region and negative deviations in the organic solvent-rich range (see Fig. 4). The positive values of  $\Delta\eta$  are smaller than for methanol and prove weaker interactions. The excess dielectric constant<sup>42</sup> and nuclear magnetic resonance<sup>43</sup> showed that in the whole composition range of the water-acetonitrile mixtures there are several structures. In the water-rich region, the acetonitrile molecules progressively occupy the cavities inside the aqueous network. In this region, the pure water structure is preserved intact, which is in agreement with the positive deviations of viscosity (see Fig. 4). The second region corresponds to the progressive disruption of water structure. The cavities are filled up to a certain level coincident with the inflexion point of  $\Delta\eta$  at  $\varphi \sim 0.4$ . The large aggregates are progressively broken into smaller entities because of a strong tendency for segregation of the water and acetonitrile molecules. This range of composition called the “microheterogeneity region” has its limit toward the acetonitrile rich region at  $\varphi \sim 0.1$ , corresponding to the observed minimum of  $\Delta\eta$ . In the region rich in the organic solvent, the existing original structure of acetonitrile is progressively disturbed by addition of water.

In the RPLC with hydro-organic mobile phases, the stationary phase is viewed as a ternary combination of bonded hydrocarbon chains, adsorbed solvent molecules and residual silanols on the silica surface.<sup>44</sup> The preferential adsorption of the less polar organic solvent on the surface of stationary phase generates another phase-layer between the mobile and the reversed phase and produces a different type of elute-solvent interaction. In this process, the concentration of organic solvent unassociated with water in the aqueous mobile phase appears to be the major factor controlling the elute retention process.<sup>45</sup> In the case of the methanol-water solutions, there are possible molecular associates of methanol, water and mixture thereof. There is evidence that with methanol as organic modifier, the adsorbed layer on the stationary phase and the bulk mobile phase have almost similar composition.<sup>45</sup> However, with acetonitrile the organic solvent-water interactions take place to a lesser extent than for methanol-water as our viscosity results show and consequently, the solvation of the stationary phase is stronger. If non-bonded silanols are present onto the stationary phase, they can contribute to the retention process by specific interactions with the components of the mobile phase and with elutes possessing functional groups.<sup>38,44</sup> The formation of stationary phase depends on the hydrogen-bonding properties of the organic solvents and on the solubility parameter of the octadecyl group. The solvents able to form hydrogen-bondings can effectively compete with water and displace it from the residual silanols. Owing to the weaker hydrogen-bonding ability of acetonitrile compared with methanol and its dispersive interaction with C<sub>18</sub>, one may admit that acetonitrile cannot displace all the

water from the residual silanols.<sup>44</sup> Therefore, the anomalous behavior of acetonitrile manifests itself through the overall retention process.

In the reversed-phase chromatography of ethoxylated surfactants, the hydrophobic moieties penetrate in-between the bonded alkyls of the fur-like stationary phase, whereas the hydrophilic PEO chains are oriented toward the polar mobile phase.<sup>27</sup> With acetonitrile as organic modifier, the alkyl chains of the stationary phase are more stretched due to their better solvation. The larger the percentage of organic solvent in the mobile phase, the larger the stretching. To explain the retention of ethoxylates, one has to consider also the contribution of the EO groups close to the alkyl chains, the polarity of the mobile phase and the solvation of the EO groups. In the mobile phase rich in methanol, the EO groups are subjected to proton-acceptor interactions with the hydroxyl groups of water and methanol and the established hydrogen bonds should justify the slight decrease of retention by increasing the PEO chain length. However, such interactions are not possible with acetonitrile where dipolar interactions are apparent. The observed by us increase of surfactant retention with the PEO chain length when the eluent is rich in acetonitrile, could be explained by considering the specific interactions between the oxyethylene chain and the accessible residual silanols. The case was previously noticed for crown ethers.<sup>46</sup> These interactions are stronger with acetonitrile than with methanol, which tends to block the silanol groups.<sup>38</sup> By increasing the water content of the eluent, the surface silanols are masked and the silanophilic interactions are attenuated, and gradually the retention behavior of the surfactants becomes regular (see Fig. 2). Therefore, the irregular behavior of ethoxylated surfactants in reversed phase systems is the result of the following factors: the shift in the conformer equilibrium to less polar conformers of the PEO chain, the change in the solvation of the PEO chain with the lowering of the water content, and the silanophilic interactions.

## CONCLUSIONS

The results obtained by us concerning the effect of methanol and acetonitrile as eluent modifiers in the reversed-phase retention of polydisperse ethoxylated nonylphenols allowed the following conclusions: The  $\log k'$  values of the surfactants decreased linearly with the methanol concentration in the eluent. The ethoxylation degree of the surfactants had a very small effect on the retention for methanol and it was more evident for acetonitrile. The inversion point of the retention was at about 20% (vol.) water for both elution systems. By examining the surface tension and viscosity of the hydro-organic eluents we collected additional data to support the retention of ethoxylates. The surface tension values decreased continuously with the content of methanol in the eluent, which agreed with the retention data. For the acetonitrile-water mixtures, the surface tension remained constant after the initial drop. The positive values of the viscosity deviation for methanol-water mixtures indicated a strong association between the alcohol and water molecules. The viscosity changes suggested a feeble interaction between acetonitrile and water. It was speculated that the acetonitrile molecules unassociated with water in the mobile phase adsorb on the stationary phase and interact with the polar EO groups close to the alkyl moiety of the surfactants. This could explain the increase of retention with the ethoxylation degree of the surfactants by increasing the acetonitrile content of the eluent.

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