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Comparison of chromatographic behaviour of oligoethylene glycol nonylphenyl ether non-ionic and anionic surfactants in reversed-phase high-performance liquid chromatography

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Abstract

A simple retention mechanism is proposed to describe the retention behaviour of oligomeric sulphated and non-sulphated surfactants of the oligoethylene glycol nonylphenyl ether type in reversed-phase systems containing ion-pairing reagents in aqueous–organic mobile phases. The equations derived using this mechanism relate the capacity factors of the individual oligomers to the number of oxyethylene units and to the concentrations of the organic solvent (2-propanol) and of the ion-pairing reagent (cetyltrimethylammonium bromide) in the mobile phase. Most capacity factors calculated using the best-fit parameters of these equations agree with the experimental values with errors of less than $\pm 5\%$ relative. The capacity factors of the anionic surfactants with various degrees of polymerization on a C_{18} column reach maximum values in the mobile phases containing 0.02 mol/l cetyltrimethylammonium bromide, while the retention of the non-ionic surfactants decreases regularly with increasing concentration of the ion-pairing reagent. The concentration of propanol affects the retention of the anionic and non-ionic surfactants in the same way, but the anionic surfactants are more strongly retained. The composition of the mobile phase can be optimized so that the sulphated and non-sulphated oligoethylene glycol nonylphenyl ethers are distinguished into two groups of peaks and the separation of the individual oligomers in the two groups is possible in a single run.

1. Introduction

Oxyethylenated alcohols, carboxylic acids and phenols are common non-ionic surfactants and their sulphated derivatives are also widely used as anionic surfactants in various industrial and household applications. Efficient separations of oxyethylenated non-ionic surfactants can be achieved by high-performance liquid chromatography (HPLC) with both normal-phase [1–11]

and reversed-phase [12–17] systems. Few reports have been published on the systematic investigation of their retention behaviour [18,19]. Much less work has been reported on the chromatographic separation of anionic sulphated derivatives than of their non-sulphated parent compounds. In addition to the separation of the sulphated from the non-sulphated fraction [20], ion-pair reversed-phase chromatography has been used to separate the individual oligomers in the sulphated and non-sulphated fractions [21–23]. Both non-sulphated and sulphated oligomers

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are eluted in order of decreasing number of oligomeric oxyethylene units in reversed-phase systems, as in chromatography on a mixed-mode reversed-phase–ion-exchange column [24], but in contrast to chromatography in normal-phase systems. Separation of sulphated oxyethylenated surfactants has also been attempted by ion-exchange chromatography on silica gel-based stationary phases [25].

Recently, we have suggested equations for the description of the simultaneous effects of the degree of polymerization, n , and of the concentration of the stronger solvent in binary mobile phases on the retention in oligomeric series, in both reversed-phase [26,27] and normal-phase [28] systems. In this work, this theory was extended to reversed-phase ion-pair chromatographic systems and conditions were established for the separation of both non-ionic oligoethylene glycol nonylphenyl ethers (OEG-NPEs) and their anionic sulphated derivatives in a single run.

2. Theoretical

Sulphated derivatives of OEGNPEs are completely ionized in aqueous–organic solutions and, like other organic and inorganic anions, are not retained in reversed-phase systems with non-polar adsorbents, unless ionic modifiers are added to the aqueous–organic mobile phase to increase the retention and make possible the separation of the ionized samples. Most common are modifiers with a large hydrophobic part and a small ionized functional group, carrying a charge opposite to that of the sample ions. The modifiers, often called ion-pairing reagents, usually show surface-active properties.

To describe chromatographic behaviour in aqueous–organic mobile phases containing ion-pairing additives, various retention models proposed so far have been reviewed recently [29]. The original “ion-pair chromatography” model based on the formation of the electroneutral associates (ion pairs) between the sample ion and the modifier ion of opposite charge [30] did not take into account the adsorption of the ionic

modifier on the surface of the non-polar column packing material. This sorption was assumed to be controlled by a Langmuir isotherm in the “dynamic ion-exchange model” [31], which yielded the same formal mathematical description of the chromatographic behaviour as the “ion-pair” model. The so-called “ion-interaction model” does not rely on the formation of ion pairs in the mobile phase [32]. Instead, the ionic modifier was assumed to control the sorption of sample ions by means of its effect on the interfacial surface tension [33]. Finally, electrostatic models have been proposed that do not take into account the formation of stoichiometric ion associates in either the mobile or stationary phase. The effect on the retention of ionic samples is explained by the sorption of the ionic modifier followed by the formation of an electrical potential over a diffuse layer adjacent to the surface of the particles of column packing material and the retention of ionic samples is attributed to the electrostatic forces in the diffuse layer [34,35]. This model leads to equations describing the influence of the concentration of the ionic modifier on the retention of ionic solutes formally similar to those applying to ion-exchange systems.

In developing a model describing the effect of the number of oligomeric units and of the concentrations of the long-chain ionic modifier [cetyltrimethylammonium bromide (CTAB)] and of the organic solvent in water–2-propanol mobile phases on the retention of sulphated derivatives of oxyethylenated nonylphenols on a C_{18} column, we had to take into account the following main features of the experimental behaviour in the system investigated: the analytes are not retained in absence of CTAB and the dependences of their retention on the concentration of CTAB show pronounced maxima. To account for the experimental behaviour, we used an approach similar to that proposed by Knox and Hartwick [31] to describe the retention of analytes in the reversed-phase system studied. However, because of the character of the compounds investigated here, the present treatment differs from theirs as follows.

- (1) The concentration of free (non-associated)

anionic analytes in the stationary phase was neglected as these compounds are eluted with the column dead volume in mobile phases that do not contain ionic additives.

(2) As the concentrations of CTAB used in this work were one order of magnitude higher than those commonly used in the ion-pair chromatography of small ions, adsorption of the cetyltrimethylammonium (CTA⁺) cations on the surface of the non-polar column,



is assumed to be better described by a Langmuir than by a linear isotherm, in agreement with the previous model [33]:

$$[\text{CTA}]_S = \frac{a[\text{CTA}]_M}{1 + b[\text{CTA}]_M} \quad (2)$$

where a (dimensionless) is the distribution ratio of CTA⁺ ions in concentrations close to infinite dilution and b (l/mol) is related to the saturation capacity q_s of the column packing material for CTA⁺ cations: $b = a/q_s$.

(3) As in the model of Knox and Hartwick [31], the formation of ion pairs of solute anions, X⁻, with CTA⁺ cations in the mobile phase was considered:



characterized by the equilibrium constant K_{IM} :

$$K_{IM} = \frac{[\text{XCTA}]_M}{[\text{X}]_M[\text{CTA}]_M} \quad (4)$$

(4) Rather than real ion exchange between sulphated OEGNPEs and Br⁻ counter ions, the association of the solute anions X⁻ with CTA⁺ cations already adsorbed in the stationary phase was assumed:



characterized by the equilibrium constant K_{IS} :

$$K_{IS} = \frac{[\text{XCTA}]_S}{[\text{X}]_M[\text{CTA}]_S} \quad (6)$$

This association can be understood as ion-pair formation in the stationary phase. The retention is enhanced as the concentration of CTA⁺ cat-

ions in the stationary phase increases. On the other hand, the formation of ion pairs in the mobile phase leads to a decrease in the retention proportional to the concentration of CTA⁺ cations in the mobile phase. Because the concentration of CTA⁺ cations in the stationary phase is proportional to the concentration in the mobile phase by means of the Langmuir isotherm, maximum retention is expected to occur at a certain concentration of CTA⁺ cations in the mobile phase.

The capacity factor $k' = (V_R/V_M - 1)$ of the sample anions, A, is defined as

$$k' = \phi K_D = \phi \cdot \frac{[\text{X}]_S + [\text{XCTA}]_S}{[\text{X}]_M + [\text{XCTA}]_M} \approx \phi \cdot \frac{[\text{XCTA}]_S}{[\text{X}]_M + [\text{XCTA}]_M} \quad (7)$$

because the free anions X⁻ are not retained in the absence of the CTA⁺ cations, so that $[\text{X}]_S = 0$. K_D in Eq. 7 is the distribution ratio, $\phi = V_S/V_M$ is the phase ratio in the column, i.e., the ratio of the volumes of the stationary and mobile phases, V_S and V_M , respectively, and V_R is the retention volume.

Combination of Eqs. 2, 4, 6 and 7 leads to the expression for k' as a function of the concentration of CTA⁺ cations in the mobile phase, $[\text{CTA}]_M = c_M$:

$$k' = \frac{\phi \cdot \frac{K_{IS}}{K_{IM}} \cdot a}{(1 + bc_M) \left(1 + \frac{1}{K_{IM}c_M} \right)} = \frac{A}{1 + Bc_M + \frac{C}{c_M}} \quad (8)$$

where $A = \phi K_{IS}a/(K_{IM} + b)$, $B = K_{IM}b/(K_{IM} + b)$ and $C = 1/(K_{IM} + b)$.

According to Eq. 8, k' decreases as either Bc_M or C/c_M increases, i.e., it increases as the saturation capacity, $q_s = a/b$, of the bonded phase for CTAB increases and as the equilibrium constant of the sorption, K_{IS} , increases, while it decreases as the constant of the ion-pair formation, K_{IM} , in

the mobile phase increases. Eq. 8 predicts maximum retention to occur at $c_M = (C/B)^{1/2}$.

It is interesting that a maximum of retention as a function of c_M is not predicted if ion-pair formation in the mobile phase is not considered and if it is assumed that the true dynamic ion exchange, i.e., the exchange between the adsorbed sample anions and Br^- counter anions, controls the retention provided that the sorption of CTA^+ cations follows the Langmuir isotherm. In the absence of a buffer in the mobile phase (the sulphated OEGNPEs are ionized over a broad pH range including neutral solutions) and as the sample ions are present in low concentrations which can be neglected, the condition of electroneutrality requires that the concentration of the Br^- anions is the same as that of the CTA^+ cations both in the mobile and in the stationary phases and consequently c_M would be involved only in the denominator of the Langmuir equation (Eq. 2) and in the equation for k' , so that a steady decrease of retention would be expected as the concentration of CTAB is increased in the chromatographic system.

To obtain appropriate retention and good separation in ion-pair reversed-phase systems, it is necessary to adjust the polarity of the mobile phase by controlling the concentration of the organic solvent as the second additive to the aqueous–organic mobile phase. In the chromatographic separation of oligomeric series such as that of sulphated OEGNPEs, the number of the repeat oligomeric units such as $-\text{O}-\text{CH}_2-\text{CH}_2-$ (oxyethylene) groups, is the most important structural factor. It has been found earlier that the dependence of the capacity factors of both non-sulphated and sulphated OEGNPEs on the number of oxyethylene groups, n , and on the volume fraction of 2-propanol, φ , in mobile phases consisting of CTAB, 2-propanol and water can be described by the equation [22]

$$\log k' = a_{0P} + a_{1P}n - (m_{0P} + m_{1P}n)\varphi \quad (9)$$

where a_{0P} , a_{1P} , m_{0P} and m_{1P} are constants independent of n and φ and relate to 2-propanol as the component of the mobile phase.

Non-sulphated OEGNPEs cannot show ionic

interactions with ionic modifiers and if a modifier such as CTAB contains a bulky non-polar part, it can affect the retention by modifying the polarity of the mobile phase and the properties of the stationary phase where it is sorbed in a similar way to the organic solvents in aqueous–organic mobile phases, so that the following equation can be assumed to apply:

$$\log k' = a_{0C} + a_{1C}n - (m_{0C} + m_{1C}n)c_M \quad (10)$$

where c_M is the molar concentration of CTAB in the mobile phase and a_{0C} , a_{1C} , m_{0C} and m_{1C} are constants independent of n and c_M and relate to CTAB as the component of the mobile phase. (At the very low concentrations of ionic modifiers used in ion-pair reversed-phase chromatography, using molar concentrations is equivalent to but more convenient than working with volume fractions. Of course, the numerical values of a_{0C} , a_{1C} , m_{0C} , m_{1C} would be different when different concentration units are used.)

If the influence of the organic solvent on retention of non-sulphated OEGNPEs is independent of that of the ionic modifier, the following retention equation can be written to describe simultaneous effects of the two mobile phase additives:

$$\log k' = a_0 + a_1n - (m_{0P} + m_{1P}n)\varphi - (m_{0C} + m_{1C}n)c_M \quad (11)$$

where m_{0P} , m_{1P} , m_{0C} and m_{1C} are constants of Eqs. 9 and 10 and relate to 2-propanol and CTAB, respectively, as the components of the mobile phase. The following relationships apply for the constants a_0 and a_1 : $a_0 = a_{0P} + m_{0C}c_M = a_{0C} + m_{0P}\varphi$, $a_1 = a_{1P} + m_{1C}c_M = a_{1C} + m_{1P}\varphi$.

Unlike non-sulphated OEGNPs, their sulphated derivatives are affected primarily by ionic interactions with CTAB, which are assumed to be more important than the possible non-polar contribution of CTAB to the retention and, if the latter can be neglected, combination of Eqs. 8 and 9 results in the following relationship for the description of the dependence of the retention of sulphated OEGNPEs on the number of oxyethylene units, n , and concentrations of

CTAB, c_M , and 2-propanol, φ , in the mobile phase:

$$\log k' = a_0 + a_1 n - (m_{0P} + m_{1P} n) \varphi - \log \left(1 + B c_M + \frac{C}{c_M} \right) \quad (12)$$

3. Experimental

Some experiments were performed using an M6000A pump, a U6K injector and an M440 UV detector operated at 254 nm (all from Waters, Milford, MA, USA), connected to a TZ 4241 line recorder and a CI 100 computing integrator (both from Laboratory Instrument Works, Prague, Czech Republic). An HP 1090M liquid chromatograph equipped with a UV diode-array detector, operated at 230 nm, an automatic sample injector, a 3DR solvent delivery system, a thermostated column compartment and a Series 7994A workstation was used for other experiments.

A stainless-steel column (300 × 3.8 mm I.D.) was packed in the laboratory with spherical octadecylsilica gel Silasorb SPH C₁₈ (7.5 μm) (Lachema, Brno, Czech Republic) using a high-pressure slurry-packing technique.

Oligoethylene glycol nonylphenyl ethers (OEGNPEs) with various declared average stoichiometric ratios of oxyethylene units to nonylphenol were obtained from Servo (Delden, Netherlands) under the commercial names Serdox NNP 4, NNP 5 and NNP 8. Oligomeric anionic surfactants were prepared by sulphation of OEGNPEs and the products prepared contained both sulphated and non-sulphated oligomers. The samples were dissolved in the mobile phase in appropriate concentrations to yield good UV detector responses.

2-Propanol of spectroscopic grade was obtained from Lachema and CTAB from Serva (Heidelberg, Germany). Water was doubly distilled in glass with addition of potassium permanganate. All the solvents were filtered using a Millipore 0.45-μm filter and the mobile phases were prepared by mixing the components in the required ratios and degassed by ultrasonication

before the use, or were prepared directly in the HP 1090M instrument from the components continuously stripped with a stream of helium.

Column dead volumes, V_M , were determined using methanol and ²H₂O as dead volume markers and refractometric detection with an R 401 differential refractometer (Waters). The retention volumes, V_R , of the members of oligomeric series were measured at different mobile phase compositions. The mean values of V_R from three repeated experiments were used for calculations of the capacity factors, $k' = (V_R/V_M - 1)$, of the individual oligomers. The identification of the peaks was performed as discussed previously [19,22]. An Adstat software package and a 386 AT personal computer were used for linear and non-linear single- and multiple-parameter regression of the experimental sets of retention data as a function of the concentrations of the mobile phase components and of the number of oligomeric units.

4. Results and discussion

Sulphated OEGNPEs are not retained on a C₁₈ column in mobile phases containing only 2-propanol and water and are eluted at the column dead volume in a single, unresolved peak, so that they can be separated from the parent non-sulphated oligomers, which are retained under these conditions. To make the separation of the individual sulphated oligomers possible, it is necessary to increase their retention by the addition of a cationic ion-pair reagent, such as CTAB. Experimental capacity factors of non-sulphated and sulphated OEGNPEs on a Silasorb SPH C₁₈ column in aqueous-organic mobile phases with various concentrations of 2-propanol and CTAB are given in Tables 1 and 2, together with the values of k' calculated from Eqs. 11 and 12 fitted to the experimental data sets. The retention ($\log k'$) decreases regularly as the number of oxyethylene units in the oligomers increases, in agreement with earlier experiments on octylsilica and octadecylsilica columns [22]. The reversed order of elution of both sulphated and non-sulphated

Table 1

Capacity factors, k' , of non-sulphated OEGNPEs with n oxyethylene units on a Silasorb SPH C_{18} (7.5 μm) column (300 \times 3.8 mm I.D.) in mobile phases containing c_M mol/l CTAB and $\varphi \cdot 10^2\%$ (v/v) 2-propanol in water

| n | Capacity factor ^a | $\varphi = 0.50$, $c_M = 0$ | $\varphi = 0.50$, $c_M = 0.005$ | $\varphi = 0.50$, $c_M = 0.02$ | $\varphi = 0.50$, $c_M = 0.04$ | $\varphi = 0.50$, $c_M = 0.08$ | $\varphi = 0.35$, $c_M = 0.04$ | $\varphi = 0.40$, $c_M = 0.04$ | $\varphi = 0.45$, $c_M = 0.04$ | $\varphi = 0.60$, $c_M = 0.04$ |
|-----|------------------------------|---------------------------------|-------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| 1 | k'_e | 3.69 | 3.47 | 3.14 | 2.81 | 2.16 | 9.76 | 6.46 | 4.24 | 1.30 |
| | k'_c | 3.63 | 3.51 | 3.18 | 2.78 | 2.13 | 9.22 | 6.18 | 4.15 | 1.25 |
| 2 | k'_e | 3.37 | 3.19 | 2.87 | 2.49 | 1.90 | 8.38 | 5.59 | 3.72 | 1.16 |
| | k'_c | 3.31 | 3.20 | 2.87 | 2.49 | 1.88 | 8.11 | 5.48 | 3.70 | 1.14 |
| 3 | k'_e | 3.06 | 2.88 | 2.58 | 2.20 | 1.65 | 7.18 | 4.85 | 3.27 | 1.03 |
| | k'_c | 3.02 | 2.91 | 2.60 | 2.24 | 1.66 | 7.14 | 4.85 | 3.29 | 1.03 |
| 4 | k'_e | 2.76 | 2.59 | 2.30 | 1.98 | 1.43 | 6.31 | 4.27 | 2.89 | 0.92 |
| | k'_c | 2.75 | 2.65 | 2.35 | 2.00 | 1.46 | 6.28 | 4.29 | 2.93 | 0.94 |
| 5 | k'_e | 2.52 | 2.37 | 2.08 | 1.76 | 1.26 | 5.54 | 3.76 | 2.57 | 0.83 |
| | k'_c | 2.51 | 2.41 | 2.12 | 1.80 | 1.29 | 5.53 | 3.80 | 2.61 | 0.85 |
| 6 | k'_e | 2.33 | 2.18 | 1.90 | 1.59 | 1.13 | 4.96 | 3.40 | 2.31 | 0.76 |
| | k'_c | 2.29 | 2.19 | 1.92 | 1.61 | 1.13 | 4.87 | 3.37 | 2.33 | 0.77 |
| 7 | k'_e | 2.14 | 2.01 | 1.75 | 1.43 | 1.02 | 4.48 | 3.11 | 2.11 | 0.69 |
| | k'_c | 2.09 | 1.99 | 1.74 | 1.45 | 1.00 | 4.28 | 2.98 | 2.08 | 0.70 |
| 8 | k'_e | 1.99 | 1.87 | 1.61 | 1.34 | 0.93 | 4.12 | 2.89 | 1.92 | 0.61 |
| | k'_c | 1.90 | 1.81 | 1.57 | 1.30 | 0.88 | 3.77 | 2.64 | 1.85 | 0.64 |

Column dead volume $V_M = 2.35$ ml.

^a k'_e = Experimental capacity factors; k'_c = capacity factors calculated from the best-fit parameters of Eq. 11: $\log k' = 2.362 - 0.0671n - 3.523\varphi - 2.720c_M + 0.054\varphi n - 0.182c_M n$.

OEGNPEs is apparent from the shift of the maxima of the mass distribution to lower retention times with increasing nominal degree of polymerization in order Serdiox NNP 4 — Serdiox NNP 5 — Serdiox NNP 8 in mobile phases containing 0.04 mol/l CTAB in 45% 2-propanol (Fig. 1).

Fig. 2 shows dependences of $\log k'$ of sulphated and non-sulphated OEGNPEs on the volume fraction, φ , of 2-propanol in aqueous-organic mobile phases containing 0.04 mol/l CTAB. The plots are well characterized by straight lines in the concentration range of 2-propanol investigated and the slopes of the individual oligomers within each series are very close to each other, which means that the oligomeric selectivity, α , is almost independent of the concentration of 2-propanol in the mobile phase. The values of α for the sulphated and non-sulphated OEGNPE series are very close to each other in a mobile phase of a given composition and the selectivity of separation of the individual oligomers increases slightly with decreasing concentration of 2-propanol in the mobile phase

(Table 3). This is due to the same oxyethylene repeat structural unit in the two series. However, both the absolute values of k' and the slopes of the $\log k'$ versus φ plots are significantly higher with sulphated than with non-sulphated OEGNPEs. It has been demonstrated earlier [22] that the differences in the slopes, as characterized by the parameter m_{op} in Eq. 9, increase with increasing size of the end-group in the oligomeric series. This is in agreement with the present experimental observations, as the end-group in sulphated OEGNPEs is much bulkier than that in the non-sulphated series, because it is contributed to not only by the sulpho group, but also by the associated bulky CTA⁺ pairing cation.

The effect of the concentration of CTAB on the retention of sulphated OEGNPEs is very different from the effect on that of the non-sulphated parent series, as is demonstrated by the dependences of $\log k'$ on c_M in Figs. 3 and 4. The plots for non-sulphated oligomers in Fig. 3 show a linear decrease, in agreement with Eq. 10, much like the dependences of $\log k'$ on the

Table 2

Capacity factors, k' , of sulphated OEGNPEs with n oxyethylene units on a Silasorb SPH C₁₈ (7.5 μm) column (300 × 3.8 mm I.D.) in mobile phases containing c_M mol/l CTAB and $\varphi \cdot 10^2\%$ (v/v) 2-propanol in water

| n | Capacity factor ^a | $\varphi = 0.50$, $c_M = 0.005$ | $\varphi = 0.50$, $c_M = 0.02$ | $\varphi = 0.50$, $c_M = 0.04$ | $\varphi = 0.50$, $c_M = 0.08$ | $\varphi = 0.40$, $c_M = 0.04$ | $\varphi = 0.45$, $c_M = 0.04$ | $\varphi = 0.60$, $c_M = 0.04$ | $\varphi = 0.65$, $c_M = 0.04$ |
|-----|------------------------------|-------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| 1 | k'_c | – | – | 7.85 | 6.61 | 26.72 | 14.37 | 2.58 | 1.48 |
| | k'_e | 6.36 | 9.04 | 8.24 | 6.31 | 25.84 | 14.59 | 2.63 | 1.48 |
| 2 | k'_c | – | 7.97 | 7.44 | 5.91 | 23.37 | 13.47 | 2.45 | 1.41 |
| | k'_e | 5.75 | 8.17 | 7.45 | 5.71 | 23.05 | 13.10 | 2.41 | 1.37 |
| 3 | k'_c | 4.83 | 7.57 | 6.76 | 5.23 | 20.53 | 12.15 | 2.26 | 1.28 |
| | k'_e | 5.19 | 7.39 | 6.73 | 5.16 | 20.55 | 11.76 | 2.21 | 1.26 |
| 4 | k'_c | 4.43 | 6.95 | 6.02 | 4.58 | 18.18 | 10.77 | 2.04 | 1.17 |
| | k'_e | 4.70 | 6.68 | 6.09 | 4.66 | 18.33 | 10.56 | 2.02 | 1.17 |
| 5 | k'_c | 4.09 | 6.30 | 5.41 | 3.99 | 15.95 | 9.54 | 1.85 | 1.08 |
| | k'_e | 4.25 | 6.04 | 5.50 | 4.22 | 16.34 | 9.48 | 1.85 | 1.08 |
| 6 | k'_c | 3.79 | 5.72 | 4.87 | 3.52 | – | 8.52 | 1.68 | – |
| | k'_e | 3.84 | 5.46 | 4.97 | 3.81 | 14.58 | 8.52 | 1.70 | 0.99 |
| 7 | k'_c | 3.59 | 5.24 | 4.42 | 3.16 | – | 7.65 | 1.56 | – |
| | k'_e | 3.47 | 4.93 | 4.50 | 3.44 | 13.00 | 7.65 | 1.56 | 0.92 |
| 8 | k'_c | – | – | 4.03 | 2.92 | – | 7.07 | – | – |
| | k'_e | 3.14 | 4.46 | 4.07 | 3.11 | 11.59 | 6.86 | 1.43 | 0.84 |
| 9 | k'_c | – | – | 3.71 | 2.57 | – | 6.33 | – | – |
| | k'_e | 2.84 | 4.03 | 3.67 | 2.82 | 10.34 | 6.16 | 1.31 | 0.78 |

Column void volume $V_M = 2.35$ ml.

^a k'_e = Experimental capacity factors; k'_c = capacity factors calculated from the best-fit parameters of Eq. 12: $\log k' = 3.718 - 0.073n - 5.022\varphi + 0.059n\varphi - \log(1 + 15.379c_M + 0.0061/c_M)$.

volume fraction of 2-propanol in Fig. 2. This supports the idea that the role of CTAB in controlling the retention of non-ionic solutes in reversed-phase systems is principally based on modifying the polarity of the mobile phase and of the surface of the column packing material.

The agreement of the experimental capacity factors with the k' values calculated from Eq. 10 fitted to the experimental data proves suitability of this equation to describe the retention of non-sulphated OEGNPEs in the chromatographic system studied (the differences between the experimental and the calculated k' values exceed 5% relative for only three out of 72 values in Table 1 and the agreement for all the k' values is better than 10% relative). This precision of calculation allows unquestionable attribution of the calculated k' to the experimental peaks of the individual oligomers if the composition of the mobile phase is varied within the range investigated in this work.

As expected, the effect of the concentration of

CTAB, c_M , on the retention of sulphated OEGNPEs is different from the effect on that of the parent non-sulphated oligomers. The plots of $\log k'$ of the individual oligomers show maxima in mobile phases containing approximately 0.02 mol/l ion-pairing reagent (Fig. 4). The points correspond to the experimental data and the curves were calculated using the best-fit parameters of the logarithmic form of Eq. 8. The differences between calculated and experimental k' values were 0.2 or less, i.e., the relative errors of the fitted data were less than 4%, which is comparable to the experimental errors in the determination of k' and demonstrates a good fit of Eq. 8 to the experimental data.

Such a maximum of retention as a function of c_M is predicted on the basis of the retention model described by Eqs. 8 and 12. Eq. 8 predicts no retention ($k' = 0$) for $c_M = 0$ and a maximum retention for $c_M = (K_{IM}b)^{-1/2}$. The occurrence of the maximum can be explained as follows: at low concentrations of CTAB, c_M , the concentration

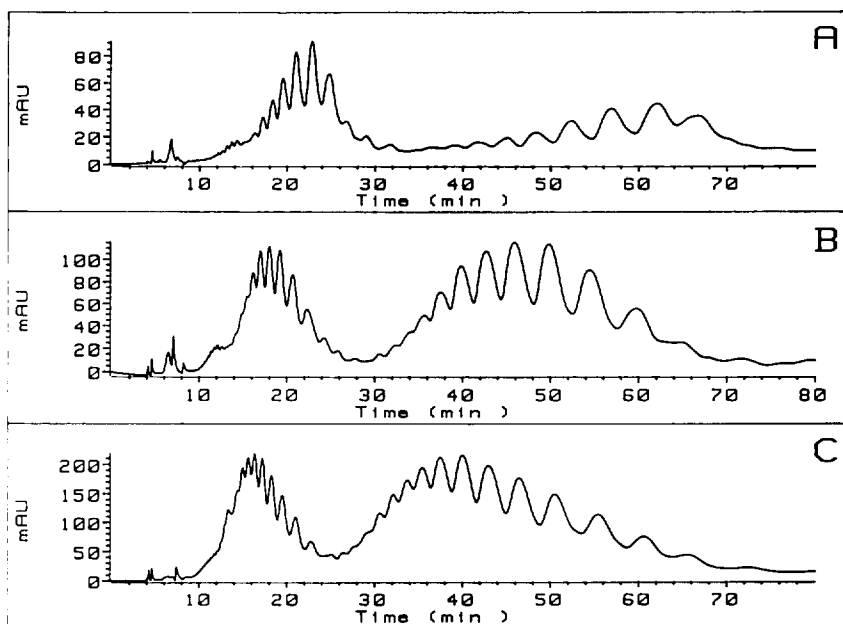


Fig. 1. Separation of partially sulphated samples of OEGNPEs with various nominal degrees of ethoxylation, (A) Serdox NNP 4, (B) Serdox NNP 5 and (C) Serdox NNP 8, on a Silasorb C_{18} SPH ($7.5 \mu\text{m}$) column ($300 \times 3.8 \text{ mm I.D.}$) with aqueous–organic mobile phases containing 45% 2-propanol and 0.04 mol/l CTAB at a flow-rate of 0.5 ml/min. Detection, UV at 230 nm. The first group of peaks belongs to non-sulphated and the second group to sulphated OEGNPEs.

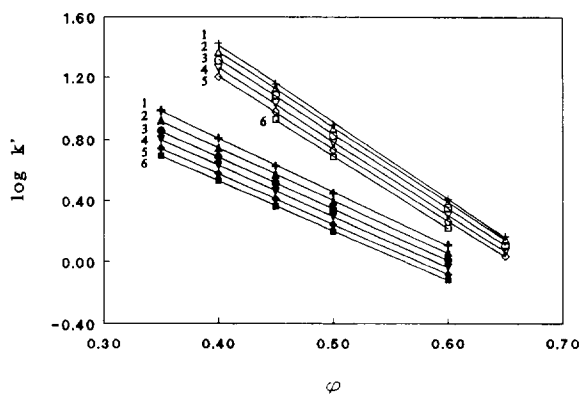


Fig. 2. Dependence of the retention (capacity factors, k') of non-sulphated (closed symbols) and sulphated (open symbols) OEGNPEs with various numbers of oxyethylene units (given by the numbers on the plots) on the volume fraction ϕ ($10^{-2}\%$ v/v of 2-propanol in aqueous–organic mobile phases containing 0.04 mol/l CTAB). Column as in Fig. 1. Points, experimental values; lines, dependences calculated from Eqs. 11 and 12 with the best-fit parameters.

of the ion-pairing reagent adsorbed in the stationary phase, c_s , increases approximately linearly with increase in c_M (steep part of the Langmuir isotherm) and the retention is enhanced because the amount of the sorbed ions of the analytes is proportional to the concentration of CTAB in the stationary phase. The increase of the amount of CTAB adsorbed with increase in c_M is less steep in the higher concentration range of CTAB, corresponding to the shallow part of the Langmuir isotherm, and its effect eventually becomes less important than the competition of CTA^+ cations in the mobile phase for the anionic solute, so that an increase in c_M causes a decrease in the retention.

The values of the parameters of Eq. 12 obtained from the best fit to the complete data set at various concentrations c_M and ϕ are given in Table 2, together with the capacity factors calculated using Eq. 12 and the best-fit parameters. The deviations of the calculated values from the experimental k' values are higher than those for the data obtained by fitting Eq. 8, because of an

Table 3

Parameters of the equation $\log k' = \log \beta + n \log \alpha$ for sulphated (series 1) and non-sulphated (series 2) OEGNPEs on a Silasorb SPH C₁₈ (7.5 μ m) column (300 \times 3.8 mm I.D.) in aqueous mobile phases with various concentrations of 2-propanol (φ) and of CTAB (c_M)

| c_M (mol/l) | φ (10 ⁻² % v/v) | Series 1 | | | Series 2 | | |
|------------------|---------------------------------------|-------------|--------------|--------|-------------|--------------|--------|
| | | Log β | Log α | R^a | Log β | Log α | R^a |
| 0.005 | | 0.778 | -0.033 | 0.9968 | 0.578 | -0.037 | 0.9985 |
| 0.02 | | 0.985 | -0.037 | 0.9984 | 0.538 | -0.042 | 0.9986 |
| 0.04 | | 0.949 | -0.043 | 0.9984 | 0.487 | -0.047 | 0.9979 |
| 0.08 | | 0.870 | -0.052 | 0.9985 | 0.379 | -0.053 | 0.9971 |
| | 0.40 | 1.481 | -0.056 | 0.9999 | 0.866 | -0.059 | 0.9966 |
| | 0.45 | 1.217 | -0.047 | 0.9974 | 0.671 | -0.051 | 0.9983 |
| | 0.50 | 0.951 | -0.043 | 0.9970 | 0.487 | -0.047 | 0.9978 |
| | 0.60 | 0.460 | -0.038 | 0.9970 | 0.154 | -0.046 | 0.9986 |

^a Correlation coefficient.

additional parameter, φ , included in Eq. 12, but are lower than 10% relative and the differences exceed 5% relative only for 10 values out of 57. This agreement suggests that Eq. 12 is suitable to describe the retention behaviour of sulphated OEGNPEs in the chromatographic system studied. As with non-sulphated OEGNPEs, the precision of calculation allows the calculated k' to be safely attributed to the experimental peaks in mobile phases with various concentrations of

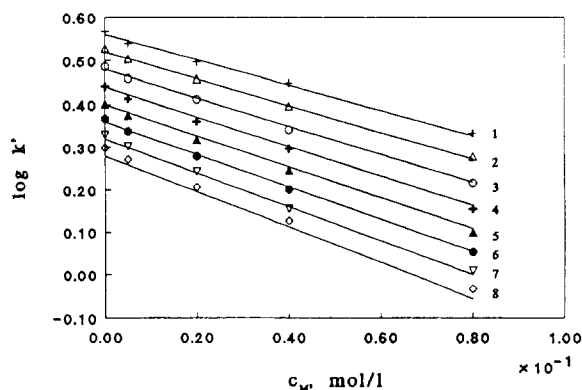


Fig. 3. Dependence of the retention (capacity factors, k') of non-sulphated OEGNPEs with various numbers of oxyethylene units (given by the numbers on the plots) on the molar concentration, c_M , of CTAB in aqueous-organic mobile phases containing 50% 2-propanol. Column as in Fig. 1. Points, experimental values; lines, dependences calculated from Eq. 11 with the best-fit parameters.

2-propanol and CTAB (with the exception of the oligomers with 6–8 oxyethylene units in a mobile phase containing 0.08 mol/l of CTAB).

The fit of a retention equation to the set of experimental data alone does not prove the validity of the underlying theoretical model in the chromatographic system studied. However, there is some additional supporting evidence that can be extracted from the present experimental data.

The constants a and b of the Langmuir iso-

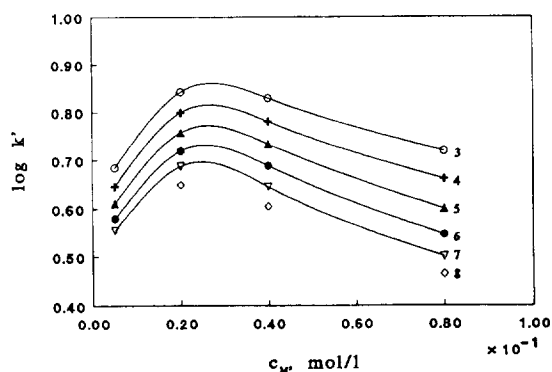


Fig. 4. Dependence of the retention (capacity factors, k') of sulphated OEGNPEs with various numbers of oxyethylene units (given by the numbers on the plots) on the molar concentration, c_M , of CTAB in aqueous-organic mobile phases containing 50% 2-propanol. Column as in Fig. 1. Points, experimental values; lines, dependences calculated from Eq. 8 with best-fit parameters.

them are not expected to depend on the number of oxyethylene units in the molecule of the analyte. Further, as the distribution between the stationary and the mobile phases in reversed-phase systems is controlled principally by the polarity and size of the sample solutes, the number of oxyethylene units, n , is likely to affect much more significantly the equilibrium constant K_{IS} of the association of the sample anions with CTA^+ cations in the stationary phase than the equilibrium constant K_{IM} of the formation of ion pairs in the mobile phase, which is controlled by the electrostatic attraction forces and depends principally on the magnitude of the charges of the interacting ions. Consequently, the number of the oligomeric units, n , is expected to affect much more significantly the constant A of Eq. 8 than the parameters B and C . Also the concentration of 2-propanol in the mobile phase is likely to affect the equilibrium constant K_{IS} and the constant A more significantly than the constant K_{IM} or the constant b of the Langmuir isotherm and consequently the constants B and C of Eq. 8. The derivation of Eq. 12 is based on the above assumptions and it is possible to calculate the values of the constants K_{IM} and b from the parameters B and C of this equation. The present set of experimental data yielded the following values of the constants: $B = 15.4 \pm 2.6$, $C = 0.00605 \pm 0.00008$, $K_{IM} = 17 \pm 3$ and $b = 148 \pm 5$ l/mol. The constants B and C were calculated also from the experimental data sets for the individual oligomers and the calculated values were almost independent of the number of oxyethylene units, n . The value of b is in reasonable agreement with $b = 312$ l/mol ($a = 436$) determined by measuring the Langmuir sorption isotherm of CTAB on a C_8 column in 30% aqueous methanol, where the experimental data agreed with the values calculated from the Langmuir isotherm with relative errors between 1.4 and 5.4% in the CTAB concentration range $1 \cdot 10^{-3}$ – $4 \cdot 10^{-3}$ mol/l [36].

The following supporting evidence for the validity of the assumptions accepted in the derivation of the present retention model can be found in the experimental data:

(1) Not only are the values of the oligomeric selectivities α in the non-sulphated and sul-

phated OEGNPE series in Table 3 very close to each other for mobile phases with a given concentration of 2-propanol, φ , but also the intercepts a_{1P} (0.067 and 0.073, respectively) and the slopes m_{1P} (0.054 and 0.059, respectively) of the dependences of $\log \alpha$ on φ are almost identical in the two series (Tables 1 and 2). Also, the selectivities α in mobile phases with a given concentration of CTAB, c_M , are very similar for the sulphated and non-sulphated OEGNPE series (Table 3). This behaviour is possible only if the logarithmic term on the right-hand side of Eq. 12 and consequently the constants K_{IM} and b (and the parameters B and C) of Eq. 8 are independent of the number of oxyethylene units, n , and φ , otherwise the intercepts and slopes of the above dependences for the two series would have different values. On the other hand, the constant K_{IS} (Eq. 6) should depend on the concentration of 2-propanol, φ , and on the number of oxyethylene units, n , much in the same way as the distribution ratio (or the capacity factor) of non-sulphated OEGNPEs (especially in the quasi-linear part of the CTAB isotherm, where the ratio $[CTA]_S/[CTA]_M$ is constant), as both the K_{IM} and the distribution of non-sulphated oligomers are controlled mainly by the polarities of the stationary and mobile phases and by the size of the analytes. Consequently, the constant K_{IM} is contributed to by the parameters a_0 , a_1 , m_0 and m_1 of Eq. 14 essentially in the same way as k' of non-sulphated OEGNPEs by the constants a_{0P} , a_{1P} , m_{0P} and m_{1P} . Hence the similarity of the intercepts and slopes of the dependences of $\log \alpha$ on φ is in agreement with the assumptions made in the derivation of the retention model.

(2) The logarithmic term in Eq. 12, $Y = \log(1 + Bc_M + C/c_M)$, calculated (a) from c_M and the constants B and C of Eq. 8 and (b) from the experimental k' and constants a_{0P} , a_{1P} , m_{0P} and m_{1P} have identical values within the limits of the experimental error (Table 4).

(3) The value of the term $a_0 - Y$ in Eq. 12 evaluated from the complete data set for the sulphated OEGNPEs ($3.72 - 0.25 = 3.47$, Tables 3 and 4) is in very good agreement with the value of the constant a_{0P} of Eq. 9 (3.46), calculated by fitting Eq. 9 to the limited data set acquired

Table 4

Comparison of the values of $Y = \log(1 + Bc_M + C/c_M)$ calculated (a) from c_M and the parameters B and C of Eq. 8 and (b) from the experimental k' and the parameters a_0 , m_0 , a_1 and m_1 of Eq. 12, $Y = a_0 + a_1n - (m_0 + m_1n)\varphi - \log k'$ [arithmetic means for various oligomers with different numbers of oxyethylene units, n , and concentrations of 2-propanol, φ ($10^{-2}\%$, v/v), \pm standard deviations] at various concentrations of CTAB in the mobile phase (c_M)

| Parameter | c_M (mol/l) | | | |
|-----------|---------------------------------------------|---------------------------------------------|----------------------------------------------|---------------------------------------------|
| | 0.005 | 0.02 | 0.04 | 0.08 |
| Y (a) | 0.36 | 0.21 | 0.25 | 0.36 |
| Y (b) | 0.37 ± 0.02 ($N = 5$) ^a | 0.19 ± 0.02 ($N = 8$) ^a | 0.25 ± 0.01 ($N = 35$) ^a | 0.38 ± 0.02 ($N = 9$) ^a |

^a Number of calculated values of Y for different n and φ .

at a constant concentration of CTAB $c_M = 0.04$ mol/l.

(4) The concentration of CTAB at which the retention maxima occur, $c_M(\text{max})$, is independent of the number of oxyethylene units, n , in sulphated OEGNPEs (Fig. 4) and can be predicted from the parameters B and C of Eq. 12: $c_M(\text{max}) = (C/B)^{1/2} = (K_{\text{IM}}b)^{-1/2} = 0.0198$ mol/l. This behaviour obviously follows from the independence of K_{IM} and b of the number of oxyethylene units assumed in the derivation of the retention model.

The selectivity of separation of the individual oligomers in both the sulphated and non-sulphated OEGNPE series increases very slightly with increasing concentration of CTAB and with decreasing concentration of 2-propanol in the mobile phase, and the absolute retention of non-sulphated OEGNPEs increases with decreasing concentrations of both CTAB and 2-propanol (Table 3). Because the absolute retention of sulphated OEGNPEs decreases with increasing concentration of 2-propanol, but shows a maximum at 0.02 mol/l CTAB, the best separation of the groups of sulphated and non-sulphated OEGNPEs and simultaneously good resolution of the individual oligomers in the two groups can be accomplished with mobile phases containing CTAB at a slightly higher concentration than that yielding maximum retention (i.e., 0.03–0.04 mol/l) and as low a concentration of 2-propanol as is acceptable from the point of view of the time of analysis (40–45%, v/v). Under these conditions, the separation of both sulphated and non-sulphated OEGNPEs in a sample mixture is

possible in a single run on a C_{18} column, such as in the examples shown in Fig. 1.

The group of non-sulphated OEGNPEs is eluted in 30 min under these conditions. As the contents of the lower oligomers decrease with increasing nominal degree of ethoxylation, the maxima of the molecular mass distributions of both sulphated and non-sulphated OEGNPEs are shifted to lower elution volumes (compare Fig. 1A and C), so that distinct resolution of the two groups is possible without significant overlapping for mixtures containing oligomers with up to 12–13 oxyethylene units. To separate mixtures of higher oligomers, the concentration of 2-propanol in the mobile phase can be decreased, but at the cost of longer separation times.

The efficiency of separation is not very high in this example, probably because of the viscosity of the mobile phase used, but it could be possibly increased with a more efficient column, if the pressure limits allow its use. Unfortunately, using less viscous organic modifiers (e.g., methanol or acetonitrile) and tetraalkylammonium salts with shorter alkyl chains failed to improve the separation because the selectivity of separation of the individual oligomers was very low in these mobile phases.

5. Conclusions

The theoretical prediction of the retention of non-ionic oligomers such as OEGNPE surfactants with different degrees of polymerization

can be extended to aqueous–organic mobile phases containing an ion-pairing reagent, the effect of which is similar to that of the concentration of the organic solvent in the mobile phase: it modifies the polarities of the mobile and stationary phases in the chromatographic system used. In these systems, it is possible to calculate the retention (k') as a function of three variables: the number of oligomeric units and the concentrations of the organic solvent and of the ion-pairing reagent in the mobile phase. The ion-pairing reagent does not affect significantly the separation selectivity of the individual oligomers and these calculations would not be of practical value if only non-ionic oligomers are separated, but predictions of this type are potentially useful when mixtures containing both ionic and non-ionic surfactants are chromatographed, as the addition of an ion-pairing reagent is necessary for separations of anionic surfactants.

Cationic ion-pairing reagents with long alkyl chains affect the retention of anionic surfactants in two different ways: the sorption of the large ion-pairing cations on the surface of the stationary phase enhances the sorption of anionic solutes in the form of ion associates at low concentrations of the ion-pairing reagents, whereas at high concentrations the competition of ion-pairing cations in the mobile phase for anionic solutes predominates and decreases the retention. Consequently, maximum retention is observed at a certain concentration of the ion-pairing reagent in the mobile phase. The effect of the concentration of the organic solvent in the mobile phase on the retention of anionic surfactants is similar to that with the non-ionic parent compounds. The retention behaviour of anionic surfactants as a function of the number of oligomeric units and of the concentrations of both the ion-pairing reagent and of the organic solvent in the mobile phase can be described by a simple equation.

Simultaneous separations of the individual sulphated and non-sulphated OEGNPE oligomers can be accomplished on a C₁₈ column with mobile phase containing 2-propanol and CTAB in water. The concentrations of 2-propanol and CTAB in the mobile phase can be selected on

the basis of the model retention equations to optimize the retention and the selectivity of separation of the individual oligomers. For this purpose, a mobile phase containing 0.04 mol/l CTAB in 45% 2-propanol was found to be the most suitable.

The possibilities of separating sulphated and non-sulphated OEGNPEs on columns packed with silica gel and polar bonded stationary phases will be discussed elsewhere [37].

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