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Chromatographic behaviour in reversed-phase high-performance liquid chromatography with micellar and submicellar mobile phases

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Abstract

Micellar HPLC can be used to separate non-ionic analytes in reversed-phase systems with aqueous mobile phases containing surfactants in concentrations higher than critical micellar concentrations (CMC). Ionic analytes are often separated by ion-pair reversed-phase chromatography in aqueous-organic mobile phases containing lower concentrations of ionic surfactants. The objective of the present work was to compare chromatographic behaviour of non-ionic solutes in micellar mobile phases and in mobile phases containing surfactants in concentrations lower than CMC (submicellar mobile phases). CMC were measured for several cationic and anionic surfactants on an octylsilica column. The Langmuir isotherm was modified to describe the distribution of the surfactants between the stationary and the mobile phases in the submicellar concentration range. Retention behaviour of polar solutes with low retention in reversed-phase systems was measured in aqueous mobile phases containing surfactants in concentrations lower and higher than CMC. According to the form of the dependence of their retention on the concentration of the surfactant in the submicellar and micellar range of mobile phase compositions, the analytes were classified into four different classes. Equations describing the retention in dependence on the concentration of surfactant in submicellar and micellar mobile phases were derived and experimentally verified. The forms of the equations are the same for all analytes, but the meanings of the equation constants depend on the compound class. The selectivity of separation of a number of polar compounds is better in submicellar than in aqueous-organic mobile phases and the efficiency of separation is improved with respect to the micellar HPLC. Further, the sensitivity of non-specific detection, e.g. with refractive index detectors, is significantly better in submicellar than in micellar mobile phases. Submicellar HPLC has been applied to separation of various types of compounds, such as lower alcohols, barbiturates, phenols and nitriles of carboxylic acids used as the intermediates in production of some herbicides.

Keywords: Mobile phase composition; Micellar liquid chromatography; Retention behaviour; Surfactants

1. Introduction

Surfactants (surface active agents) are often used as mobile phase additives in liquid chromatography. When present in concentrations higher than so-called critical micellar concentration (CMC), several surfactant molecules form aggregates — micelles. The liquid phase is then comprised of the aqueous phase and of the micellar pseudo-phase. In reversed-phase chromatography, the surfactant becomes significantly adsorbed on the surface of the non-polar stationary phase (usually of the bonded alkylsilica gel type). Solutes can partition between the aqueous, the micellar and the stationary phases, which is the basis of the micellar liquid chromatography (pseudo-phase liquid chromatography).

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For a long time, charged surfactants have been used in HPLC as mobile phase additives in concentrations below the CMC, where micelles do not exist. Anionic surfactants can form ion pairs with cationic solutes and vice versa. The ion pairs behave much like uncharged compounds, which improves possibilities of their separation in so-called ion-pair chromatography systems [1,2]. According to another view, a charged surfactant was assumed to be adsorbed and to affect the distribution of charged solutes between the stationary and the mobile phases mainly by ionic interactions in the stationary phase. The ions of a solute with similar charges as the adsorbed surfactant ions are repulsed and the retention decreases, whereas the solute ions oppositely charged are attracted by the surfactant ions and the retention increases with increasing concentration of the surfactant in the mobile phase.

Not only ionic solutes are affected by the presence of surfactants, but the retention of non-charged neutral compounds was found to decrease as the concentration of adsorbed surfactant on the alkylsilica stationary phase is increased [3]. This behaviour was attributed to lowering the interfacial tension between the adsorbed phase and the bulk liquid phase as the concentration of the surfactant in the mobile phase c_s increases. A decrease in the distribution coefficient of a neutral compound results. In addition to this effect, the adsorption of the surfactant on the bonded phase changes its properties, which may affect the retention of neutral analytes [4].

The following equation was suggested to describe the dependence of the capacity factor k' of an oppositely charged solute on the concentration of ionic surfactant in the mobile phase c_s assuming Langmuir adsorption isotherm of the surfactant and neglecting the interfacial tension effects [5]:

$$\ln k' = b_1 + \frac{b_2 \cdot c_s}{(c_s + b_3)} \tag{1}$$

where b_1 is $\ln k'$ in the absence of the surfactant, the constant b_2 relates to the extent of the interaction between the adsorbed solute and surfactant and b_3 characterizes the energy of adsorption of the surfactant.

To describe the retention of an uncharged neutral solute, another equation was suggested [6]:

$$\ln k' = a_1 - a_2 \cdot \ln\left(1 + \frac{c_s}{a_3}\right) \tag{2}$$

Here, a_1 is $\ln k'$ in the absence of the surfactant, the constant a_2 depends on the maximum surface coverage of the surfactant and on the molar surface area of the solute and a_3 has the same meaning as b_3 in Eq. 1.

The retention in micellar mobile phases has been studied previously by several groups of workers and the retention equations suggested to describe the retention in dependence on the concentration of micelles in the mobile phase, $c_m = c_s - \mathrm{CMC}$, can be written in the following form [7,8]:

$$\frac{1}{k'} = \frac{K_{MW}}{\Phi \cdot [S]_S \cdot K_{SW}} \cdot c_m + \frac{1}{\Phi \cdot [S]_S \cdot K_{SW}} = A + B \cdot c_m \tag{3}$$

where $[S]_S = Q_{CMC}$ is the concentration of the surfactant in the stationary phase, K_{SW} and K_{MW} are the equilibrium constants of the association of the analyte in the aqueous phase with the surfactant in the stationary phase and with the micelles, respectively. $\Phi = V_S/V_M$ is the phase ratio, i.e. the ratio of the volumes of the stationary (V_S) and of the mobile (V_M) phases and A and B are formal constants.

Formal validity of Eq. 3 has been verified experimentally. In agreement with Eq. 3, the retention of uncharged solutes and of compounds with a charge opposite to that of the surfactant was found to decrease as the concentration of the micelles in the mobile phase increases, if the analytes form inclusion complexes with the micelles. However, solutes carrying similar charge as the surfactant are excluded from the micelles and their retention increases as c_m is increased. This implies negative values of the constant K_{MW}, which would have no physical meaning [9] because the distribution constant is defined as the ratio of the concentrations in two phases and consequently the underlying model is not consistent.

In most previous works, the retention behaviour of analytes was studied either in so-called ion-pair or in micellar systems. With few exceptions [6,10,11] little attention was paid to the investigation of the re-

tention of uncharged solutes over the surfactant concentration range covering both micellar and submicellar mobile phases. The objective of the present work was to compare and to describe the retention of various compounds in reversed-phase systems with surfactants in both micellar and submicellar mobile phases and to study possibilities of using submicellar instead of micellar mobile phases for separations of polar compounds.

2. Theoretical

2.1. Sorption isotherms of surfactants

When the concentration of a solute in the solution in contact with the adsorbent is being increased, its concentration in the stationary phase usually steeply rises at first, but then the increase of the adsorbed amount gradually diminishes as it approaches the maximum (saturation) capacity of the adsorbent. The adsorption of various solutes in reversed-phase systems is most often described by the Langmuir isotherm (Eq. 4) or by equations derived from the models departing from it [12]:

$$Q = \frac{a \cdot c}{1 + b \cdot c} \tag{4}$$

Here, Q and c are the concentrations of the solute in the stationary and in the mobile phases, respectively, a is the distribution coefficient at infinite dilution and b is related to the saturation capacity of the adsorbent Q_s for the solute: $Q_s = a/b$. According to the Langmuir isotherm, the saturation capacity would be achieved at infinitely high concentration in the mobile phase.

Experimental adsorption isotherms of surfactants in reversed-phase systems have profiles different from the isotherms of simple organic compounds. An abrupt break is observed on the isotherm and the adsorbed amount remains approximately constant if the concentration of the surfactant in bulk solution is higher than the critical micellar concentration (CMC) [6,13,14]. This is explained by the fact that the concentration of free molecules of ionic surfactants in bulk solution is constant and equal to CMC, whereas only the concentration of micelles increases as the total concentration of the surfactant is raised and the micelles obviously are not adsorbed. (This need not apply to non-ionic surfactants.)

The adsorption isotherm is usually described by the Langmuir equation, which however sometimes has been found to describe inaccurately the experimental behaviour of the surfactants [15]. To overcome this problem, a heterogeneous adsorbent surface with sites of different binding abilities was considered, or the Langmuir isotherm was modified to include the term respecting the effects of the surface potential [16].

The previous models do not consider the break in the increase of the adsorbed amount at CMC. To respect this phenomenon, we modified the Langmuir isotherm by introducing the maximum adsorbed concentration corresponding to $c = \mathrm{CMC}$:

$$Q_{\mathrm{CMC}} = \frac{a' \cdot \mathrm{CMC}}{1 + b' \cdot \mathrm{CMC}};$$

$$b' = \frac{a'}{Q_{\mathrm{CMC}}} - \frac{1}{\mathrm{CMC}} \tag{5}$$

where a', b' are constants of the modified Langmuir isotherm and $a' = k_0'/\Phi$, where k_0' is the capacity factor of the surfactant at infinite dilution and $\Phi = V_S/V_M$ is the phase ratio, i.e. the ratio of the volumes of the stationary and of the mobile phase in the column. Introducing a', b' back to the Langmuir equation (Eq. 4) we obtain:

$$Q = \frac{a' \cdot c_s}{1 + \left[\frac{a'}{Q_{\mathrm{CMC}}} - \frac{1}{\mathrm{CMC}}\right] \cdot c_s} \tag{6}$$

This equation applies for $c_s < \mathrm{CMC}$ and $Q = Q_{\mathrm{CMC}}$ for $c_s \geq \mathrm{CMC}$. CMC can be determined directly and only one batch or dynamic experiment at the plateau of the isotherm is necessary to find Q_{CMC}. a', the distribution coefficient of the surfactant at infinite dilution, is then the only unknown quantity in Eq. 6.

2.2. Retention in submicellar mobile phases

To describe the retention of neutral compounds and of weak acids and bases in submicellar mobile phases, we assume that the adsorption of the surfac-

tant in the stationary phase is controlled by modified Langmuir isotherm (Eq. 6). An ionic surfactant S can form ion pairs XS with solutes X carrying opposite charges and solvation associates or other complexes with neutral analytes both in the mobile and in the stationary phases:

$$X_M + S_M \rightleftarrows XS_M\,, \quad X_S + S_S \rightleftarrows XS_S \qquad (7a,7b)$$

characterized by the equilibrium constant K_M:

$$K_M = \frac{[XS]_M}{[X]_M \cdot [S]_M} \qquad (8)$$

and K_S:

$$K_S = \frac{[XS]_S}{[X]_S \cdot [S]_S} \qquad (9)$$

introducing Eq. 6, Eq. 8 and Eq. 9 into the definition equation for k' of the solute we obtain:

$$\begin{aligned} k' &= \Phi \cdot \frac{[X]_S + [XS]_S}{[X]_M + [XS]_M} \\ &\cong \frac{V_S}{V_M} \cdot \frac{[X]_S + [X]_M \cdot [S]_S \cdot K_S}{[X]_M \cdot (1 + [S]_M \cdot K_M)} \\ &= \frac{k_0' + (k_0' \cdot b' + k_0' \cdot K_S \cdot a') \cdot c_s}{1 + (K_M + b') \cdot c_s + b' \cdot K_M \cdot c_s^2} \end{aligned} \qquad (10)$$

where k_0' is the capacity factor in the absence of the surfactant. We have suggested earlier a similar equation to describe the retention of anionic surfactants on alkylsilica columns in mobile phases containing cationic surfactants as the additives, where the concentration of non-associated analytes in the stationary phase can be neglected [17].

If there is no association or if the concentration of possible associates of the analytes with the surfactant can be neglected with respect to the concentration of free analyte molecules in the mobile phase, Eq. 10 is simplified to:

$$k' = k_0' \cdot \left(1 + \frac{a' \cdot K_S \cdot c_s}{1 + b' \cdot c_s}\right) \qquad (11)$$

On the other hand, $K_S = 0$ for compounds that do not form associates in the stationary phase. In this case Eq. 10 is also simplified:

$$\begin{aligned} k' &= \Phi \cdot \frac{[X]_S}{[X]_M \cdot (1 + K_M \cdot [S]_M)} \\ &= k_0' \cdot \frac{1}{1 + c_s \cdot K_M} \end{aligned} \qquad (12)$$

As the molecules can be associated to the surfactant much in the same way in the mobile as in the stationary phase, zero or very low values of K_M can be expected when $K_S = 0$ and k' is expected not to depend significantly on c_s.

It is not possible to apply the above approach to the retention of solutes that are repulsed by the molecules of surfactant, such as compounds with a similar charge as the surfactant. We suggest here another approach to characterize the effect of the concentration of the surfactant on the retention of these analytes. As the repulsion forces hinder molecules of a solute to come into close contact with the molecules of the surfactant, a part of the stationary phase with adsorbed surfactant becomes inaccessible to the solutes. This means that only a part V_S of the real volume of the stationary phase, V_{SO}, affects the phase ratio in the column. To first approximation, the inaccessible part of the volume of the stationary phase can be assumed to be directly proportional to the concentration of the adsorbed surfactant, $[S]_S$ so that we obtain for V_S:

$$V_S = V_{SO} - f_S \cdot [S]_S \cdot V_{SO} = V_{SO} \cdot (1 - f_S \cdot [S]_S) \qquad (13)$$

Here, f_S is a proportionality constant which depends both on the solute and on the surfactant.

It is not likely that a solute is repulsed from the stationary phase by the adsorbed surfactant and at the same time is associated with the surfactant in the mobile phase because of the inherently similar nature of the interaction forces between the molecules in the two phases. Should this be the case, the retention equation (Eq. 10) can be modified to respect these phenomena by setting $[XS]_S = 0$ and introducing Eq. 13 for V_S. This adaptation results in the substitution of the term $k_0' \cdot K_S$ in Eq. 10 by $-k_0' \cdot f_S$.

If there is no association in the mobile phase and the solute is repulsed from the stationary phase, the

retention equation is obtained by substituting Q for $[S]_S$ from Eq. 6 and introducing Eq. 13 into the definition equation for the capacity factor:

$$k' = \frac{V_S \cdot [X]_S}{V_M \cdot [X]_M} = \frac{V_{S0} \cdot (1 - f_S \cdot [S]_S) \cdot [X]_S}{V_M \cdot [X]_M}$$
$$= k'_0 \cdot \left(1 - \frac{a' \cdot f_S \cdot c_s}{1 + b' \cdot c_s}\right) \quad (14)$$

where k'_0 is the capacity factor in the system without surfactant.

Theoretically, we could use a similar approach to describe the retention of the solutes that are repulsed from the stationary phase, but form associates in the mobile phase. However, this behaviour is not likely to occur because the interaction forces between the molecules of the analyte on one hand and of the surfactant on the other should be similar regardless of the character of the phase.

Eq. 14 can be transformed to the linear relationship

$$Y = \frac{c_s}{1 - k'/k'_0} = \frac{1}{a' \cdot f_S} + \frac{b'}{a' \cdot f_S} \cdot c_s$$
$$= \alpha + \beta \cdot c_s \quad (15)$$

where Y has the dimensions of concentration, but no real physical interpretation.

Eq. 11 can be transformed to the following equation formally similar to Eq. 15, but with different meaning of constants α, β:

$$Y = \frac{c_s}{1 - k'/k'_0} = -\frac{1}{a' \cdot K_S} - \frac{b' \cdot c_s}{a' \cdot K_S}$$
$$= \alpha + \beta \cdot c_s \quad (16)$$

Similar transformation can be applied to Eq. 12:

$$Y = \frac{c_s}{1 - k'/k'_0} = \frac{1}{K_M} + c_s = \alpha + \beta \cdot c_s \quad (17)$$

Linear dependence of the quantity Y on the concentration of the surfactant in the mobile phases predicted by Eqs. 15–17 for various types of compounds can be fitted to the experimental data sets in submicellar mobile phases to determine the constants α and β. From the values of the constants, it would be possible to distinguish between the different types of retention behaviour. If the isotherm parameters a', b' and the capacity factor of the solute in pure water, k'_0, are known, it is principally possible to determine the constants K_M, K_S, or f_S, of Eq. 11, Eq. 12 and Eq. 14 in the system studied.

Eq. 17 predicts $\beta = 1$. However, the experimental constant β close to 1 cannot be considered as a final proof that Eq. 17 controls the retention of a given analyte, because β can be close to 1 also if $b' \approx a' \cdot f_S$ in Eq. 15 derived assuming that the solutes are excluded from the stationary phase in the presence of the surfactant.

2.3. Retention in micellar mobile phases

We employed Eq. 3 to describe the retention of analytes that can form associates or complexes both with the micelles and with the surfactant adsorbed in the stationary phase. In some specific cases, this equation should be modified. If the analyte does not form an associate with the micelles, $K_{MW} = 0$ and the retention is independent of the concentration of micelles in the mobile phase, c_m; $k' = \Phi \cdot Q_{CMC} \cdot K_{SW}$.

For the retention of compounds that are repulsed by the molecules of the surfactant from the stationary phase, but can form associates with the micelles, we obtain the following equation for the capacity factor, with the aid of Eq. 13:

$$k' = \frac{V_S \cdot [X]_S}{V_M \cdot ([X]_M + [XM]_M)}$$
$$= \frac{V_{S0} \cdot [X]_S \cdot (1 - f_S \cdot Q_{CMC})}{V_M \cdot [X]_M \cdot (1 + K_{MW} \cdot c_m)} = k'_0 \cdot \frac{1 - f_S \cdot Q_{CMC}}{1 + K_{MW} \cdot c_m} \quad (18)$$

where $[X]_S$, $[X]_M$ are the concentrations of the free solute in the stationary and in the bulk mobile phases, $[XM]_M$ is the concentration of the solute in the micellar phase and k'_0 is the capacity factor in the absence of the surfactant.

In addition to the inaccessibility of a part of the

volume of the stationary phase, also a part of the volume of the mobile phase may be inaccessible to some solutes if the interaction forces with the micelles are repulsive. To first approximation, the inaccessible part is assumed to be directly proportional to the concentration of the micelles, c_m:

$$V_M = V_{M0} \cdot [1 - f_{mic} \cdot (c_s - CMC)] = V_{M0} \cdot (1 - f_{mic} \cdot c_m) \quad (19)$$

Because the concentration of the adsorbed surfactant is independent of c_m in micellar mobile phases, $[S]_S = Q_{CMC}$, we obtain Eq. 20 to describe the retention in micellar mobile phases:

$$k' = \frac{V_S \cdot [X]_S}{V_M \cdot [X]_M} = \frac{V_{S0} \cdot [X]_S \cdot (1 - f_S \cdot Q_{CMC})}{V_{M0} \cdot [X]_M \cdot (1 - f_{mic} \cdot c_m)} = k'_0 \cdot \frac{1 - f_S \cdot Q_{CMC}}{1 - f_{mic} \cdot c_m} \quad (20)$$

Here, k'_0 is the capacity factor in the absence of the surfactant. Eq. 20 predicts increasing k' as the concentration of micelles is increased, in agreement with the behaviour observed with some compounds.

Eq. 18 can be transformed to Eq. 21, formally similar to Eq. 3 applying for the compounds that form associates with the micelles:

$$\frac{1}{k'} = \frac{1}{k'_0 \cdot (1 - f_S \cdot Q_{CMC})} + \frac{K_{MW} \cdot c_m}{k'_0 \cdot (1 - f_S \cdot Q_{CMC})} = A + B \cdot c_m \quad (21)$$

The same approach can be applied also to Eq. 20 describing the retention of compounds that are excluded from the micelles:

$$\frac{1}{k'} = \frac{1}{k'_0 \cdot (1 - f_S \cdot Q_{CMC})} - \frac{f_{mic} \cdot c_m}{k'_0 \cdot (1 - f_S \cdot Q_{CMC})} = A + B \cdot c_m \quad (22)$$

The constants k'_0 and f_S in Eq. 21, Eq. 22 and in Eq. 14 applying in submicellar phases should have the same values.

If neither associates are formed nor analytes are excluded from the stationary phase in the presence of the surfactant, but associates are formed with micelles, Eq. 3 is modified as follows:

$$k' = \frac{V_S \cdot [X]_S}{V_M \cdot ([X]_M + [XM]_M)} = k'_{CMC} \cdot \frac{1}{1 + K_{MW} \cdot c_m} \quad (23)$$

and

$$\frac{1}{k'} = \frac{1}{k'_{CMC}} + \frac{K_{MW}}{k'_{CMC}} \cdot c_m = A + B \cdot c_m \quad (24)$$

where k'_{CMC} is the capacity factor in the mobile phase containing the surfactant in concentration c_s = CMC.

Finally, if analytes are neither associated nor excluded from the stationary phase in the presence of the surfactant, but are excluded from the micelles the retention equations acquire the following forms:

$$k' = \frac{V_S \cdot [X]_S}{V_{M0} \cdot [X]_M \cdot (1 - f_{mic} \cdot c_m)} = k'_{CMC} \cdot \frac{1}{1 - f_{mic} \cdot c_m} \quad (25)$$

and

$$\frac{1}{k'} = \frac{1}{k'_{CMC}} - \frac{f_{mic}}{k'_{CMC}} \cdot c_m = A + B \cdot c_m \quad (26)$$

In all the cases discussed, the reciprocal value of the capacity factor is a linear function of the concentration of micelles, like in systems described by Eq. 3. However, the constants A and B have different meanings depending on the interactions controlling the retention, as discussed above.

Some very non-polar solutes such as long-chain alkylbenzenes have very low affinities to the aqueous phase where their concentrations are close to zero, so that they are distributed virtually only between the micelles and the stationary phase. The description of the retention would be simpler in this case, as only a two-phase equilibrium can be considered. However, this case is not very important in practice, as this situation usually leads to low efficient separations with a very large peak spread.

3. Experimental

3.1. Reagents and sample compounds

Mobile phases were prepared by dissolving hexadecyltrimethylammonium bromide (CTAB, 99%+, Janssen Chimica, Beerse, Belgium or Serva, Heidelberg, Germany), or sodium dodecyl sulphate (SDS, Aldrich, Milwaukee, WI, USA) in water (double-distilled in glass with potassium permanganate and sodium hydrogencarbonate) in required proportions.

Sample compounds listed in Tables 2 and 3 were obtained from Lachema, Brno, Czech Republic, in reagent grade purities and were dissolved in the mobile phase to provide adequate detector response. Compounds with low solubilities in aqueous mobile phases were dissolved in mobile phases after addition of a few percent of methanol. The concentrations of the UV-absorbing compounds listed in Tables 2 and 3 in the samples injected (5 μl) were in the range between 0.01–0.1%, so that induced micellization of the surfactant by the sample solutes was not likely to occur.

3.2. Determination of critical micellar concentrations (CMC)

CMC values of the surfactants were determined using a conductimetric method described earlier [18]. An OK-104 conductivity meter (Radelkis, Budapest, Hungary) equipped with a Metrohm conductometric cell (Metrohm, Herishau, Switzerland) was used for the determination of CMC of CTAB and SDS. A solution of 0.0078 mol/l CTAB or of 0.0516 mol/l SDS in water was gradually added from the burette to 50 ml of water in a 250 ml beaker, thermostatted at 25°C, and the conductivity was measured after each addition of the surfactant solution. A 'titration curve' was constructed as the plot of the conductivity versus the concentration of the surfactant in the 'titrated' solution, taking into account necessary corrections for changing volumes after each addition. The CMC was determined as the point of intersection of the straight line parts of the conductimetric 'titration curves' below and above CMC. The conductivity meter was calibrated using a standard solution of 0.001 mol/l KCl (analytical grade, Lachema. Brno, Czech Republic).

3.3. Sorption isotherms

Sorption isotherms of surfactants were measured by the frontal chromatography method [19,20] using two M 6000A pumps (Waters-Millipore, Milford, MA, USA) controlled by an M660 gradient programmer (Waters) to increase stepwise the concentration of the surfactant in water. The mixed solution of the surfactant was pumped through a short stainless steel sorption column, 24 mm × 3.9 mm I.D. (Waters), packed in the laboratory with Silasorb SPH C8, 7.5 μm spherical octylsilica material (Lachema, Brno) connected to a LCD 2040 variable wavelength detector (ECOM, Prague, Czech Republic) set to 224 nm (for the isotherms of CTAB) or to a CDLC 1 conductimetric detector (Laboratory Instrument Works, Prague, Czech Republic, for the isotherms of SDS). Water and the solutions of the surfactants used in this method were filtered through 0.45-μm membrane filters (Millipore, Milford, MA, USA) and degassed by ultrasonication prior to the use.

One pump (A) delivered pure water and the second one (B) aqueous solution of the surfactant containing 0.003 mol/l CTAB or 0.015 mol/l SDS. The composition of the mixed solution was changed in 5% or 10% steps from 0% to 99% B at a constant sum of the flow-rates of the pumps A and B set at 3 ml/min. The concentration of the surfactant in the effluent from the sorption column was continuously monitored by the detector whose signal was registered using a TZ 4200 line recorder (Laboratory Instrument Works, Prague, Czech Republic). Time was allowed for the stabilization of the detector response after each concentration change. In each step the surfactant concentration in the stationary phase was calculated from the integral mass balance equation, using the experimental retention volume (inflection point on the breakthrough curve), corrected for the volume of the tubing between the mixing point of the liquid streams A and B and the column top. Always the new freshly filled column was taken for measuring each of CTAB or SDS sorption isotherms.

3.4. Elution data

A HP 1090M liquid chromatograph (Hewlett-Pac-

kard, Palo Alto, CA, USA) equipped with a DR 5 solvent delivery system, a diode-array detector and a data workstation was used to acquire the elution volumes used for the determination of the capacity factors of sample compounds. Stainless steel columns, 300 mm × 4.2 mm I.D. or 300 mm × 3.6 mm I.D., packed in the laboratory with Silasorb SPH C8, 7.5 μm (Lachema, Brno), were used for the experiments at higher concentrations of CTAB and a microcolumn, 24 mm × 3.9 mm I.D., packed with the same sorbent for the experiments in pure water or in mobile phases with low concentrations of CTAB, where large retention volumes had been expected. For the experiments with SDS, a stainless steel column, 300 mm × 3.6 mm I.D., or a microcolumn, 24 mm × 3.9 mm I.D. (for strongly retained compounds) were used. Mobile phases were prepared by mixing water (double-distilled in glass with potassium permanganate) and surfactant and were filtrated through 0.45-μm membrane filters prior to use. The flow-rate of the mobile phases was kept at 1 ml/min during the work both with analytical columns and with the microcolumn.

The column used was first equilibrated with the most concentrated mobile phase and then the retention volumes V_R of all test compounds were measured. In the subsequent sets of experiments, the concentrations of the surfactants in the mobile phases were decreased. Freshly prepared columns were employed for each of the sets of experiments with mobile phases containing either CTAB or SDS. To calculate the capacity factors, $k' = V_R/V_0 - 1$, the dead volumes V_0 of the columns used were determined after injection of D_2O with refractometric detection using an R 401 Differential Refractometer (Waters).

4. Results and discussion

To verify the retention models described in the theoretical part, the values of the critical micellar concentrations and the parameters of the adsorption isotherms of the surfactants should be known. The CMC of CTAB 1.3 mmol/l and of SDS 8.4 mmol/l in water determined by conductimetric method agree with earlier published values of 8.2 mmol/l for SDS [21] and 1.3 mmol/l for CTAB [22]. The sorption isotherms of the surfactants on a Silasorb C8 column determined in the submicellar and micellar concentrations using the frontal analysis method are shown in Fig. 1. The parameters of the Langmuir (Eq. 4) and of the modified Langmuir (Eq. 5) isotherms are given in Table 1.

The experimental isotherms show expected profiles with plateaus corresponding to constant concentrations of adsorbed surfactant, Q_{CMC}, for micellar concentrations above CMC. This is in agreement with Ref. [23]; however, it differs from the results found by Berthod et al. [6], who found increasing sorption of CTAB on a C8 column even at the concentrations above CMC. They also observed higher Q_{CMC} for CTAB than for SDS, contrary to our present results. These discrepancies could be possibly caused by a lower content of the residual silanol groups in the Silasorb C8 endcapped material, as the silanol groups may become ionized and may show anion-exchange properties resulting in a stronger sorption of the CTAB cations.

The surface coverage of the adsorbent was calculated from the experimental values of Q_{CMC}, the mass of the adsorbent in the column, the molar volumes of the surfactants and the specific surface of the adsorbent given by the manufacturer, assuming spherical shape of the surfactant molecules (Table 1). The results correspond to 60–70% coverage of

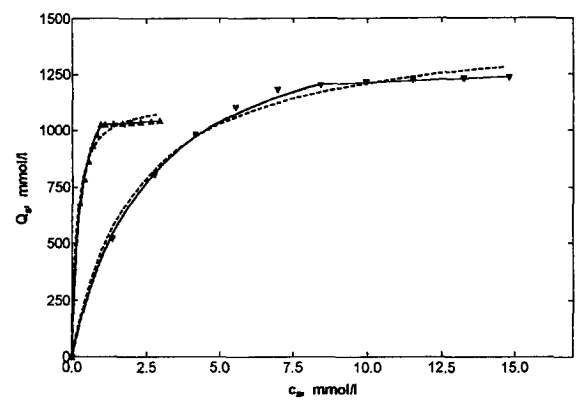

Fig. 1. Sorption isotherms of CTAB and SDS on a Silasorb SPH C8, 7.5 μm (23 mm × 3.9 mm I.D. microcolumn) in water. ▲, experimental values for CTAB; ▼, experimental values for SDS; full bold lines, modified Langmuir isotherm according to Eq. 6; dashed lines, Langmuir isotherm according to Eq. 6, full thin lines, plateau at c_s > CMC.

Table 1
Parameters of Langmuir (Eq. 4) and modified Langmuir isotherm (Eq. 6) of CTAB and SDS on a Silasorb SPH C8, 7.5 μm, 24 mm × 3.9 mm I.D. column; specific surface area of sorbent 600 m^2/g, $V_M = 0.197$ ml, $V_S = 0.075$ ml, $\Phi = V_S/V_M = 0.38$

	Surfactant	
	CTAB	SDS
CMC (mol/l)	0.0013	0.0084
a from Eq. 4	6222	684
b (l/mol) from Eq. 4	6100	465
Q_S (mol/l) from Eq. 4	1.020	1.471
k'_0 from Eq. 4	2364	260
Q_{CMC} (mol/l)	1.025	1.205
a' from Eq. 6	4958	605
b' (l/mol) from Eq. 6	3838	384
$k'_0 = a' \cdot \Phi$	1884	230
amt. sorbed (mol)	7.9×10^{-5}	9.3×10^{-5}
% covered	60	60

the surface, which indicates that a monomolecular layer of the adsorbed surfactant is formed even in micellar mobile phases. Fig. 1 shows improved fit of the modified isotherm (Eq. 6) to the experimental data in comparison with the unmodified Langmuir isotherm (Eq. 4).

The Silasorb C8 column has about 20% higher maximum sorption capacity Q_{CMC} for SDS than for CTAB, which can be attributed to higher molar volume of CTAB (0.361 l/mol) [23] than of SDS (0.285 l/mol) [24]. However, higher maximum sorption capacity for SDS may be also affected by approximately six times higher CMC of SDS as compared with CTAB. The capacity factor of CTAB at infinite dilution ($\Phi \cdot a'$) is approximately eight times higher than the corresponding value of SDS, probably because of bulkier alkyl in the molecule of CTAB.

The retention behaviour of neutral compounds, weak acids (phenols, barbiturates) and weak bases (aniline, pyridine) was studied. The acids and bases were present in the free forms and their dissociation was negligible under the chromatographic conditions used, so that no ion-pair formation could occur with the oppositely charged ions of the surfactant. However, different proportions of non-polar, dipole–dipole and proton donor–acceptor interactions between the surfactants and the sample molecules are expected for neutral compounds and for weakly acidic or basic analytes, with specific effects on the retention and on the separation selectivity.

Tables 2 and 3 show experimental capacity factors of the test solutes measured in submicellar and in micellar mobile phases containing 0–30 mmol/l CTAB and 0–80 mmol/l SDS. The plots of k' versus the concentrations of surfactants in the submicellar and micellar ranges of the mobile phase show different patterns according to which of the compounds tested were classified into the four classes A–D. The retention either increases or decreases as c_s is increased in submicellar mobile phases and increases, decreases or remains approximately constant as the concentration of micelles increases in micellar mobile phases. Figs. 4–9 illustrate this behaviour.

We found excellent agreement between the experimental capacity factors in submicellar mobile phases and the values calculated using Eq. 10 and experimental values of k'_0, a', b'. However, the parameters of the regression equation suggested negative values of the equilibrium constants K_S and K_M for some compounds, which means that the underlying model is not realistic with these compounds. Therefore we fitted linear regression Eqs. 15–17 to the experimental data sets and we found good linearity for all the compounds tested (see examples in Fig. 2). The best-fit parameters of the equations (Eqs. 15–17) in submicellar phases are listed in Tables 4 and 5. The mean standard deviations were 13% for the parameters α and 6% for the parameters β.

From the constants α and β of the regression lines

Table 2
Experimental and calculated capacity factors for tested compounds in submicellar and micellar mobile phases with CTAB on Silasorb SPH C8 columns (for details, see Experimental; *capacity factors on 24 mm × 3.9 mm I.D. microcolumn)

Compound		$c_s \times 10^3$ (mol/l)				$c_{mic} \times 10^3$ (mol/l)			
		0	0.2	0.5	1	1.7	3.7	8.7	28.7
Barbital	k'_{exp}	35.9*	10.5*	5.04	3.10	2.70	2.60	2.46	1.72
	k'_{calc}		10.4	5.24	3.06	2.74	2.63	2.38	1.73
Allobarbital	k'_{exp}	104.5*	31.5*	18.6	9.23	8.08	7.50	7.02	4.67
	k'_{calc}		33.1	16.7	9.41	8.08	7.67	6.80	4.69
Aprobarbital	k'_{exp}	184.6*	51.8*	29.8*	14.5*	12.1	11.1	9.89	6.19
	k'_{calc}		54.8	27.1	15.1	13.1	12.1	10.1	6.16
Phenobarbital	k'_{exp}	201.4*	142.6*	100.0*	52.8*	36.6*	31.6*	21.2	10.7
	k'_{calc}		142.8	94.8	54.7	36.8	31.2	22.6	10.7
Theobromine	k'_{exp}	23.5*	2.12	0.36	0.34	0.24	0.23	0.22	0.17
	k'_{calc}		1.63	0.63	0.27	0.24	0.23	0.22	0.17
Theophylline	k'_{exp}	38.2*	7.20	3.86	2.21	2.08	2.10	2.11	2.11
	k'_{calc}		7.32	3.63	2.19	2.09	2.09	2.10	2.11
Caffeine	k'_{exp}	45.7*	5.12	2.65	2.28	1.71	1.65	1.49	0.97
	k'_{calc}		4.70	2.86	2.21	1.75	1.65	1.45	0.97
Phenol	k'_{exp}	13.2	37.8*	48.2*	56.1*	47.7*	43.2*	39.1*	24.0*
	k'_{calc}		37.2	49.0	55.9	49.2	45.7	38.9	24.3
Pyrocatechol	k'_{exp}	5.53	26.5*	26.8*	46.7*	44.9*	39.7*	35.5*	25.6*
	k'_{calc}		25.6	28.0	46.4	41.8	39.9	35.9	25.6
Resorcinol	k'_{exp}	6.65	24.4*	31.8*	41.7*	35.1*	33.3*	30.0*	17.2*
	k'_{calc}		23.0	33.8	41.0	35.9	33.3	28.3	17.5
Hydroquinone	k'_{exp}	2.83	7.47	10.3	12.4	11.6	11.2	10.4	7.41
	k'_{calc}		7.46	10.4	12.4	11.8	11.3	10.2	7.45
Phloroglucinol	k'_{exp}	1.90	6.73	9.59	10.5	9.63	8.75	8.20	5.13
	k'_{calc}		6.95	9.26	10.6	9.64	9.06	7.87	5.16
Aniline	k'_{exp}	12.1	11.4	11.3	11.0	11.2	11.4	11.9	12.9
	k'_{calc}		11.5	11.2	11.0	11.2	11.3	11.6	12.8
Acetophenone	k'_{exp}	111.6*	53.6*	31.6*	25.3*	22.7	21.7	19.8	14.6
	k'_{calc}		51.8	33.6	24.8	22.5	21.6	19.7	14.5
Benzonitrile	k'_{exp}	56.5*	35.2*	24.9*	22.1	20.4	19.2	18.3	13.8
	k'_{calc}		34.0	26.3	22.5	20.2	19.6	18.1	13.9
Nitrobenzene	k'_{exp}	55.0*	48.6*	44.2*	40.1*	36.0*	32.6*	30.2*	20.9*
	k'_{calc}		48.7*	44.0	40.2	35.4	33.7	30.0	20.9
Methomyl	k'_{exp}	47.0*	6.58*	3.43	3.41	2.20	2.15	2.12	1.85
	k'_{calc}		5.69	3.90	3.26	2.20	2.17	2.10	1.85
Pyridine	k'_{exp}	14.0	4.78	2.58	1.83	1.93	2.25	2.76	4.21
	k'_{calc}		4.67	2.67	1.79	2.11	2.20	2.45	4.45

the constants K_S, K_M or f_S were calculated using the values of a', b' and k'_0 determined in independent experiments — Tables 4 and 5. Using these constants, capacity factors of the individual classes of analytes were calculated from Eq. 11, Eq. 12 and Eq. 14) and are compared with experimental k' in Tables 2 and 3.

Most constants α and β of the regression lines were positive and the constants β were close to 1, which means that Eq. 12 can be possibly used to describe the retention assuming that the formation of the associates of the analytes in stationary phases does not occur. For a few compounds, $\beta > 1$ was found, which indicates that the analyte can be repulsed from the stationary phase in the presence of the surfactant and that the retention is controlled by Eq. 14. However, this may apply also for the compounds with $\beta \cong 1$, as discussed in the theoret-

Table 3
Experimental and calculated capacity factors for tested compounds in submicellar and micellar mobile phases with SDS on Silasorb SPH C8 columns (for details, see Experimental; *capacity factors on 24 mm × 3.9 mm I.D. microcolumn)

Compound		$c_s \times 10^3$ (mol/l)					$c_{mic} \times 10^3$ (mol/l)			
		0	1	3	5	10	11.6	21.6	41.6	71.6
Barbital	k'_{exp}	35.9*	20.2*	9.90*	7.14*	4.70	4.19	4.19	3.97	3.65
	k'_{calc}		19.4	10.5	7.41	4.57	4.25	4.14	3.94	3.67
Allobarbital	k'_{exp}	104.5*	59.0*	30.8*	21.6*	13.6*	10.9	10.2	8.62	7.31
	k'_{calc}		57.8	31.4	22.0	13.3	10.9	10.1	8.72	7.28
Aprobarbital	k'_{exp}	184.6*	103.7*	55.1*	39.4*	24.8*	18.6	16.9	13.2	10.7
	k'_{calc}		102.3	55.9	39.6	24.4	18.6	16.5	13.5	10.6
Phenobarbital	k'_{exp}	201.4*	115.5*	66.9*	45.9*	28.2*	19.0	17.2	12.5	9.41
	k'_{calc}		116.6	65.1	46.2	28.2	19.4	16.4	12.6	9.36
Theobromine	k'_{exp}	23.5*	6.45	3.11	1.77	0.92	0.92	0.91	0.93	0.97
	k'_{calc}		6.74	2.78	1.75	0.90	0.91	0.92	0.93	0.94
Theophylline	k'_{exp}	38.2*	10.7	5.08	3.10	1.75	1.59	1.55	1.54	1.52
	k'_{calc}		11.0	4.70	3.07	1.74	1.56	1.55	1.54	1.52
Caffeine	k'_{exp}	45.7*	18.8	11.7	7.87	4.39	3.74	3.50	3.24	2.92
	k'_{calc}		20.8	10.5	7.37	4.61	3.68	3.53	3.25	2.91
Phenol	k'_{exp}	13.2	12.3	10.9	9.84	8.15	7.83	7.58	7.08	6.41
	k'_{calc}		12.3	10.9	9.88	8.11	7.87	7.56	7.07	6.41
Pyrocatechol	k'_{exp}	5.53					5.03	5.04	5.02	5.01
	k'_{calc}						5.04	5.04	5.03	5.01
Resorcinol	k'_{exp}	6.65	4.08	2.38	1.78	1.30	1.24	1.33	1.39	1.49
	k'_{calc}		3.99	2.40	1.82	1.28	1.23	1.26	1.33	1.45
Hydroquinone	k'_{exp}	2.83	1.42	0.91	0.58	0.38	0.40	0.43	0.50	0.62
	k'_{calc}		1.50	0.82	0.59	0.38	0.40	0.43	0.49	0.63
Phloroglucinol	k'_{exp}	1.90	0.82	0.54	0.26	0.10	0.12	0.15	0.25	1.06
	k'_{calc}		0.93	0.43	0.26	0.11	0.12	0.15	0.23	1.37
Acetophenone	k'_{exp}	111.6*	87.3*	64.8*	53.3*	39.7*	27.6*	23.2*	18.2*	13.9*
	k'_{calc}		87.7	64.4	52.9	39.9	27.2	23.4	18.4	13.9
Benzonotrile	k'_{exp}	56.5*	45.4*	35.9*	29.8*	21.9*	18.3*	16.4*	13.9*	11.8*
	k'_{calc}		46.1	35.1	29.2	22.2	17.9	16.5	14.1	11.7
Nitrobenzene	k'_{exp}	55.0*	49.6*	41.4*	36.7*	28.9*	23.3*	20.8*	16.7*	13.0*
	k'_{calc}		49.5	41.7	36.5	28.8	23.4	20.7	16.7	13.0
Methomyl	k'_{exp}	47.0*	24.3*	13.4*	9.77*	5.40*	4.95*	4.89*	4.64*	4.33*
	k'_{calc}		25.0	13.3	9.22	5.52	4.98	4.86	4.64	4.34

ical part. The analytes with positive constants α and β were attributed to the classes B, C and D.

In submicellar mobile phases with CTAB, the retention of phenols increases with increasing concentration of the surfactant and both α and $\beta < 1$, which can be explained by more significant role of the association of the analytes with the surfactant in the stationary phase than in the mobile phase, as suggested by Eq. 11 and Eq. 16. This group of compounds belongs to the class A.

The retention of most compounds in micellar mobile phases decreases as the concentration of micelles is increased, which means that these solutes can associate with the micelles. These analytes were attributed to the compound class A (compounds forming associates in the stationary phase, retention Eq. 3) or B (compounds either non-associating in (Eq. 24) or excluded from (Eq. 21) the stationary phase). The constants A and B in Tables 4 and 5 were determined by linear regression of the dependencies of $1/k'$ on c_m with the mean standard deviations of 3% for the best-fit parameters A and 7% for the best-fit parameters B (see examples in Fig. 3). The values of the constants K_{MW}, K_{SW}, f_S

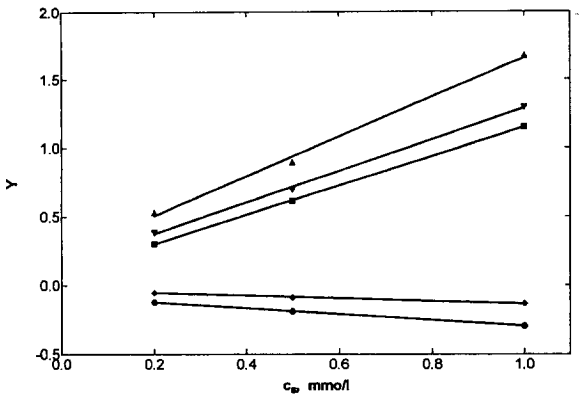

Fig. 2. Dependence of $Y = c_s/(1 - k'/k'_0)$ values (from Eqs. 15–17) on the submicellar concentration, c_s, of CTAB in water as the mobile phase. Column: Silasorb SPH C8, 7.5 μm. Compounds: ■, pyridine; ▼, acetophenone; ◆, pyrocatechol; ●, hydroquinone; ▲, benzonitrile.

and f_{mic} of Eq. 3, Eq. 21 and Eq. 24 calculated from the constants A, B using the values of k'_0 and Q_{CMC} determined in independent experiments are also given there.

k' of theobromine and of theophylline in mobile phases containing SDS and of theobromine, theophylline and pyrocatechol in systems with CTAB are independent of the concentration of the micelles, as the regression constant B in Eq. 3 and the equilibrium constant K_{MW} are close to zero. These compounds belong to class C.

The retention of aniline and of pyridine in micellar mobile phases with CTAB and of polyhydroxybenzenes in micellar mobile phases containing SDS increases with increasing concentration of micelles. Negative values of the constant B of Eq. 3 indicate that these compounds are excluded from the mi-

Table 4
Constants of the retention equations (Eqs. 27 and 28) of analytes in different classes in CTAB containing mobile phases (the constant α is in mol/l, the constants B, $K_{SW} = K_S \cdot k'_0/\Phi$, K_S, K_{MW}, K_M, f_S are in l/mol, the constants β, A are dimensionles; $\Phi = 0.38$; r is correlation coefficient)

Class A	k'_0	$\alpha \times 10^6$	β	r	A	B	r	K_{SW} (mic)	K_S (sub)	K_{SW} (sub)	–
Phenol	13.2	−60	−0.249	0.999	0.019	0.77	0.999	99.5	3.375	101.7	
Pyrocatechol	5.53	−35	−0.101	0.997	0.023	0.56	0.998	88.7	5.784	88.7	
Resorcinol	6.65	−31	−0.096	0.991	0.026	1.08	0.996	116.4	6.403	79.9	
Hydroquinone	2.83	−79	−0.218	0.999	0.082	1.82	0.998	27.7	2.564	20.6	
Phloroglucinol	1.90	−39	−0.179	0.998	0.098	3.34	0.996	23.2	5.129	21.4	
Class B	k'_0	$\alpha \times 10^3$	β	r	A	B	r	f_S (mic)	f_S (sub)	K_{MW} (mic)	K_M (sub)
Benzonitrile	56.5	0.213	1.446	0.998	0.048	0.84	0.997	0.62	0.64	17.5	4690
Nitrobenzene	55.0	1.278	2.444	0.999	0.027	0.73	0.997	0.32	0.23	27.0	782
Caffeine	45.7	0.016	1.035	0.999	0.543	16.83	0.998	0.94	1.20	31.0	62890
Methomyl	47.0	0.016	1.059	0.999	0.449	3.14	0.995	0.93	1.18	7.0	63290
Barbital	36.0	0.078	1.015	0.999	0.351	7.91	0.996	0.90	1.05	22.5	12820
Allobarbital	104.5	0.091	1.008	0.999	0.118	3.32	0.997	0.90	1.15	28.1	10990
Aprobarbital	184.6	0.083	1.006	0.999	0.071	3.18	0.987	0.90	1.06	44.8	12010
Phenobarbital	201.4	0.517	0.856	0.992	0.023	2.45	0.999	0.76	0.61	106.5	1930
Acetophenone	111.6	0.145	1.141	0.999	0.043	0.90	0.999	0.77	0.84	20.9	6900
Class C	k'_0	$\alpha \times 10^3$	β	r	A	B	r	K_{MW} (sub)	–	–	–
Theobromine	23.5	0.016	0.996	0.999	4.06	0.05	0.976	63690			
Theophylline	38.2	0.044	1.017	0.999	0.478	−0.17	0.648	22730			
Class D	k'_0	$\alpha \times 10^3$	β	r	A	B	r	f_S (mic)	f_S (sub)	f_{mic} (mic)	–
Aniline	12.2	1.631	9.19	0.989	0.090	−0.42	0.983	0.098	0.086	4.7	
Pyridine	14.0	0.089	1.059	0.999	0.489	−9.21	0.947	0.099	0.085	18.8	

Table 5
Constants of the retention equations (Eqs. 27 and 28) of analytes in different classes in SDS containing mobile phases (the constant α is in mol/l, the constants B, K_{SW}, K_S, K_{MW}, K_M, f_S are in l/mol, the constants β, A are dimensionless; $\Phi = 0.38$; r: is correlation coefficient)

Class B	k'_0	$\alpha \times 10^3$	β	r	A	B	r	f_S (mic)	f_S (sub)	K_{MW} (mic)	K_M (sub)
Phenol	13.2	13.99	1.204	0.998	0.122	0.474	0.999	0.31	0.22	3.9	71
Acetophenone	111.6	3.460	1.210	0.999	0.030	0.588	0.999	0.58	0.50	19.6	289
Benzonitrile	56.5	4.214	1.227	0.998	0.050	0.499	0.996	0.54	0.46	10.0	237
Nitrobenzene	55.0	8.771	1.227	0.999	0.036	0.574	0.999	0.41	0.30	15.9	114
Methomyl	47.0	1.113	1.022	0.999	0.195	0.496	0.997	0.74	0.85	2.5	898
Caffeine	45.7	0.802	1.032	0.999	0.257	1.214	0.996	0.76	0.94	4.7	1246
Barbital	36.0	1.139	1.032	0.999	0.228	0.620	0.985	0.73	0.83	2.7	879
Allobarbital	104.5	1.211	1.025	0.999	0.083	0.760	0.998	0.73	0.82	9.2	826
Aprobarbital	184.6	1.211	1.031	0.999	0.046	0.680	0.997	0.73	0.82	14.8	826
Phenobarbital	201.4	1.348	1.028	0.999	0.041	0.920	0.998	0.73	0.80	22.4	742
Class C	k'_0	$\alpha \times 10^3$	β	r	A	B	r	K_M (sub)	–	–	–
Theobromine	23.5	0.402	1.000	0.999	1.100	−0.503	0.934	2488			
Theophylline	38.2	0.397	1.008	0.999	0.639	0.257	0.997	2519			
Pyrocatechol	5.53	–	–	–	0.198	0.017	0.878	–			
Class D	k'_0	$\alpha \times 10^3$	β	r	A	B	r	f_S (mic)	f_S (sub)	f_{mic} (mic)	–
Resorcinol	6.65	1.411	1.097	0.999	0.838	−2.061	0.967	0.68	0.75	2.46	
Hydroquinone	2.83	1.085	1.048	0.999	1.640	−14.7	0.998	0.72	0.85	5.23	
Phloroglucinol	1.90	1.018	0.958	0.998	9.420	−121.6	0.995	0.78	0.93	12.8	

celles. For these compounds, attributed to the class D, constants f_S and f_{mic} were calculated instead of K_{MW}, K_{SW} from the regression constants A and B of Eq. 22.

The classification of the analytes into the classes A–D according to their retention behaviour in micellar and submicellar mobile phases can be summarized as follows.

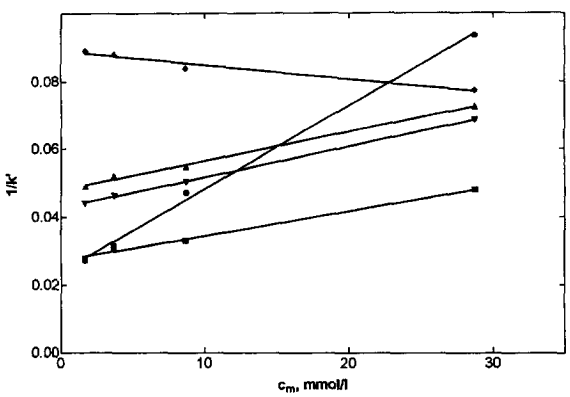

Fig. 3. Dependence of reciprocal value of k' (from Eq. 3, Eq. 21 and Eq. 24) on the micellar concentration, c_m, of CTAB in water as the mobile phase. Column as in Fig. 2. Compounds: ■, nitrobenzene; ▼, acetophenone; ◆, aniline; ●, phenobarbital; ▲, benzonitrile.

4.1. Class A

Analytes form associates with the surfactant in the stationary phase and with the micelles, but the association in the aqueous mobile phase is less significant. The retention increases in submicellar phases as the concentration of the surfactant, c_s, is increased and decreases in micellar phases as the concentration of the micelles, c_m, is raised. Examples of this behaviour in mobile phases containing CTAB are shown in Fig. 4. Eq. 11 and Eq. 16 control the retention in submicellar phases ($\alpha < 0$, $\beta < 0$) and Eq. 3 in micellar phases ($A > 0$, $B > 0$). The constants $K_{SW} = K_S \cdot k_0/\Phi$ in submicellar phases should have the same values as the constants K_{SW} in micellar phases. Approximate agreement between the two equilibrium constants was found experimentally with phenolic compounds in CTAB containing systems, where the differences were less than 10% with phenol, pyrocatechol and phloroglucinol and 25–30% with hydroquinone and resorcinol (Table 4).

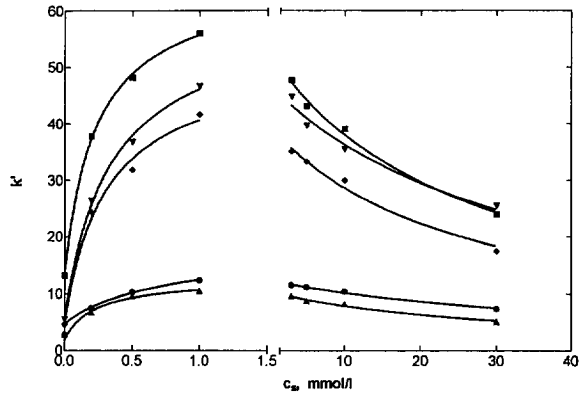

Fig. 4. Retention of phenols (class A) in submicellar (0–1 mmol/l) and micellar (3–30 mmol/) mobile phases containing CTAB in water. Column as in Fig. 2. Compounds: ■, phenol; ▼, pyrocatechol; ◆, resorcinol; ●, hydroquinone; ▲, phloroglucinol. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

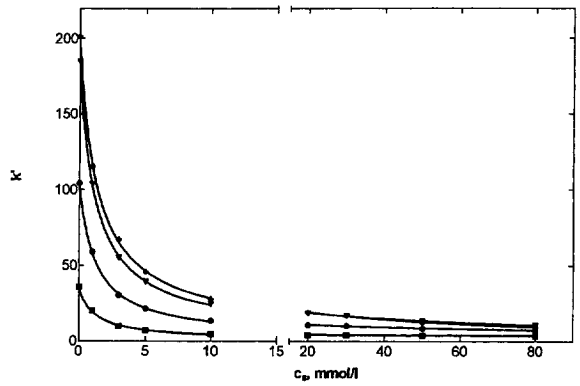

Fig. 6. Retention of barbiturates (class B) in submicellar (0–10 mmol/l) and micellar (20–80 mmol/l) mobile phases containing SDS in water. Column as in Fig. 2. Compounds: ■, barbital; ●, allobarbital; ◆, aprobarbital; ▼, phenobarbital. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

4.2. Class B

Analytes either do not form associates in the stationary phase or are excluded from it in the presence of surfactant and do not associate with the surfactant in the mobile submicellar phases, but are associated in the micelles. The retention decreases both in submicellar and in micellar mobile phases as c_s or c_m are increased. Figs. 5 and 6 show examples

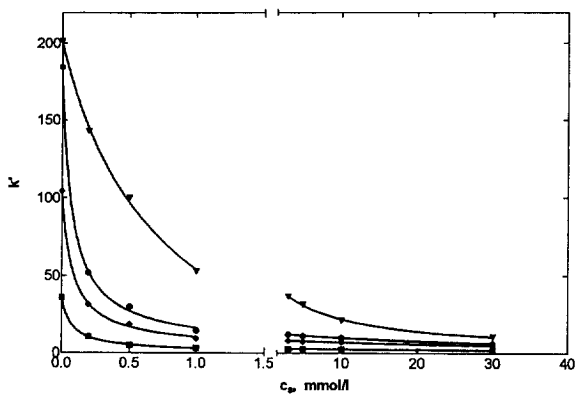

Fig. 5. Retention of barbiturates (class B) in submicellar (0–1 mmol/l) and micellar (3–30 mmol/) mobile phases containing CTAB in water. Column as in Fig. 2. Compounds: ■, barbital; ◆, allobarbital; ●, aprobarbital; ▼, phenobarbital. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

of this behaviour in mobile phases with CTAB and with SDS, respectively. The retention is controlled by Eq. 12, Eq. 17 and Eq. 14, Eq. 15 in submicellar ($\alpha > 0$, $\beta \geq 1$) and by Eq. 18, Eq. 21) or Eq. 23, Eq. 24) in micellar ($A > 0$, $B > 0$) systems. The constants β calculated using Eq. 12) are given in Tables 4 and 5. The retention model is supported by approximate agreement of the constants f_S measured in micellar and in submicellar systems (differences lower than 25% were found experimentally — Tables 4 and 5). Analytes with $\beta \approx 1$ are not necessarily excluded from the stationary phase and may form associates in the mobile phase characterized by the constant K_M. Compounds of class B may show $\beta \approx 1$ also if $b' = a' \cdot f_S$ and the retention data do not allow one to distinguish the analytes that do not associate with the surfactants in the stationary phase from those that are excluded.

4.3. Class C

Analytes do not form associates in the stationary phase in the presence of surfactants, neither they associate with the micelles. The retention decreases as the concentration of the surfactant is increased in the submicellar phase, but is independent of the concentration of micelles in micellar systems. It means that $B \approx 0$ in Eq. 3 and that Eq. 12 and Eq. 17 or Eq. 14 and Eq. 15 control the retention in

submicellar phases ($\beta \approx 1$). Here again, the behaviour in submicellar systems alone does not allow one to distinguish between the non-associating solutes and those excluded from the stationary phase if $b' = a' \cdot f_S$, as in class B. Examples of this behaviour in mobile phases with SDS are shown in Fig. 7. The constants f_S for theobromine and theophylline determined in micellar phases agree with those found in submicellar phases (Tables 4 and 5).

4.4. Class D

The retention of analytes in submicellar phases decreases as c_s is increased, but it increases in micellar mobile phases as c_m is raised as the analytes are excluded from the micelles. The constant $B < 0$ in micellar phases and the retention equation (Eq. 20) applies if the analytes are excluded from the stationary phase, or Eq. 25 describes the retention behaviour if the analytes neither form associates nor are excluded from the stationary phase in the presence of surfactants. In submicellar mobile phases, either no associates are formed or the analytes are excluded from the stationary phase, as in classes B and C. The retention data alone do not allow one to distinguish between the two cases. Aniline and pyridine in CTAB containing mobile phases (Fig. 8) and resorcinol, hydroquinone and phloroglucinol in systems with SDS (Fig. 9), i.e. weak bases in the systems with a cationic surfactant and weak acids in the systems with an anionic surfactant, belong to this class. Here, repulsive forces between the molecules of the analyte and of the surfactant are likely to control the retention, so that the exclusion from the stationary phase is probable. The values of the constants f_S determined in submicellar mobile phases agree with corresponding constants determined from the data in micellar systems (Tables 4 and 5).

In Tables 2 and 3, the experimental k' in sub-

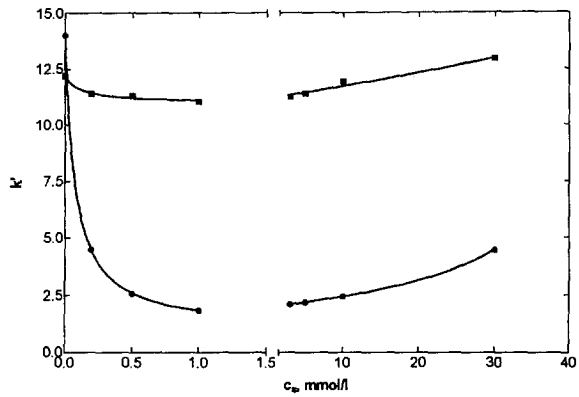

Fig. 8. Retention of aniline (■) and pyridine (●) (class D for CTAB) in submicellar and micellar mobile phases containing CTAB in water. Column as in Fig. 2. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

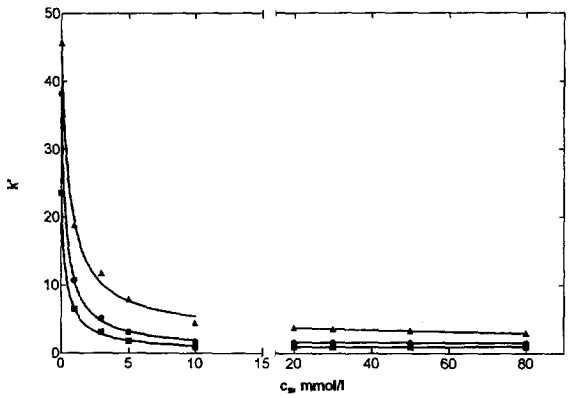

Fig. 7. Retention of theobromine (■), theophylline (●) and caffeine (▲) (classes B and C) in submicellar and micellar mobile phases containing SDS in water. Column as in Fig. 2. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

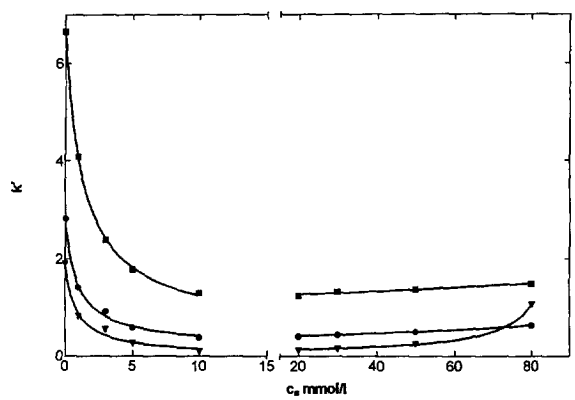

Fig. 9. Retention of phenols (class D) in submicellar and micellar mobile phases containing SDS in water. Column as in Fig. 2. Compounds: ■, resorcinol; ▼, phloroglucinol; ●, hydroquinone. Eq. 27 (in submicellar range) and Eq. 28 (in micellar range), respectively, are fitted to the experimental data.

micellar mobile phases are compared with capacity factors calculated using the equation

$$k' = k'_0 \cdot \left(1 - \frac{c_s}{\alpha - \beta \cdot c_s}\right) \quad (27)$$

obtained by slight modification of Eqs. 15–17. The tables also compare the experimental data in micellar systems with k' calculated from Eq. 3, Eq. 21, Eq. 22, Eq. 24, Eq. 26 adapted to the form

$$k' = \frac{1}{A + B \cdot c_m} \quad (28)$$

The constants α, β used for the calculations were determined by linear regression of the dependencies of Y on c_s (Eqs. 15–18) and the constants A, B by linear regression of the dependencies of $1/k'$ on c_m (Eq. 3, Eq. 21, Eq. 22, Eq. 24 and Eq. 26). The values of the correlation coefficients were better than 0.99–0.999, except for class C compounds in micellar phases where k' values are independent of c_m.

Good agreement was found between the experimental capacity factors and k' calculated using best-fit parameters of Eq. 27 and Eq. 28. The differences between the calculated and the experimental k' were lower than 5% in most cases, with only two values for theobromine and two for methomyl exceeding 10% in submicellar mobile phases containing CTAB. The advantage of this approach is that the retention of various types of compounds can be described by one two-parameter equation in submicellar systems and by another two-parameter equation in micellar mobile phases, regardless of the class of compounds. The relation of the parameters of the regression equations to the constants characterizing the distribution of a solute between the phases depends on the class to which the compound belongs. As discussed in preceding paragraphs, some of these constants should have the same values in submicellar as in micellar systems. Comparison of these constants may be helpful for better understanding of the chromatographic behaviour over the full concentration range of the surfactant in the mobile phase.

Different effects of the surfactant in submicellar and in micellar concentrations on the retention of various solutes makes it possible to use separations in submicellar mobile phases as a complement to micellar chromatography. The selectivity of separation in submicellar mobile phases may differ significantly from the selectivities in aqueous-organic mobile phases or in micellar mobile phases, which can be utilized to improve the resolution in specific separation problems. Submicellar mobile phases may offer several additional advantages with respect to the separation in micellar systems:

(1) The retention of neutral solutes is usually higher in submicellar than in micellar mobile phases, which can make submicellar chromatography more suitable for some polar solutes. As can be seen from Figs. 5–9, adjusting concentration of the surfactant in the submicellar range can have a much more significant effect on the retention and on separation selectivity of a number of compounds than the control of the concentration of the micelles in micellar chromatography.

(2) The efficiency of separation (column plate number) is often better in submicellar than in micellar mobile phases.

(3) If polar compounds that do not absorb light in the UV region are separated using refractometric detection, submicellar chromatography offers much better sensitivity of detection. The refractive index of a micellar mobile phase does not change significantly when molecules of sample compound penetrate into the micelles, so that concentrated samples must be injected to obtain a useful response of an RI detector. In submicellar mobile phases, the solute molecules are not imbedded into the micelles and the sensitivity of detection is similar to that in aqueous-organic mobile phases.

(4) Last, but not least, submicellar mobile phases are less expensive than the micellar ones, because of the lower concentrations of surfactants used.

On the other hand, submicellar mobile phases are less suitable for gradient elution, because of a strong dependence of the adsorbed amount of a surfactant on its concentration in the mobile phase, which can make re-equilibration after the end of the run lengthy and tedious. In micellar mobile phases, the concentration of the surfactant in the stationary phase is independent of the concentration of the micelles in the mobile phase.

Chromatographic behaviour in micellar and submicellar mobile phases can be illustrated by several examples.

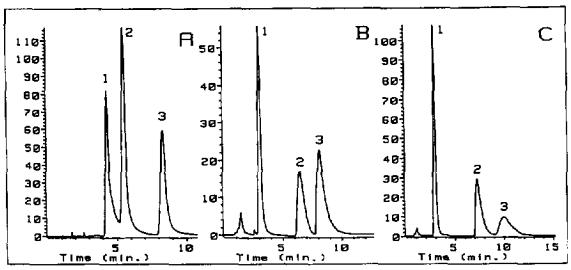

Fig. 10. Separation of theobromine (1), theophylline (2) and caffeine (3) in aqueous-organic (A), micellar (B) and submicellar (C) mobile phases. Column: Silasorb SPH C8, 7.5 μm (300 mm × 3.6 mm I.D.); mobile phase methanol–water 30:70 (v/v) (A), 0.02 mol/l CTAB in water (B), 0.002 mol/l CTAB in water (C); flow-rate 1 ml/min, detection UV, 254 nm, temperature 25°C. Detector response in milliabsorbance units.

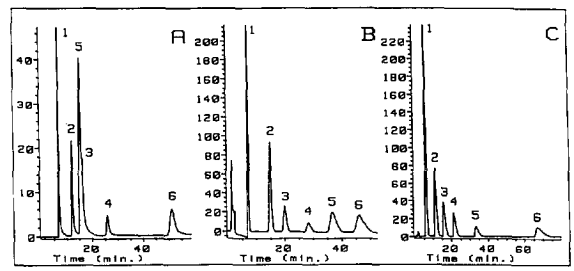

Fig. 11. Separation of barbiturates in aqueous-organic (A), micellar CTAB (B) and micellar SDS (C) mobile phases. Compounds: barbital (1), allobarbital (2), aprobarbital (3), butobarbital (4), phenobarbital (5), pentobarbital (6). Column: Silasorb SPH C8, 7.5 μm (300 mm × 3.6 mm I.D.); mobile phase methanol–water 30:70 (v/v) (A), 0.02 mol/l CTAB in water (B), 0.03 mol/l SDS in water (C); flow-rate 1 ml/min, detection UV, 240 nm, temperature 25°C. Detector response in milliabsorbance units.

Fig. 10A–C compare the separation of three xanthine alkaloids, caffeine, theophylline and theobromine in aqueous-methanolic mobile phases and in micellar and submicellar mobile phases containing CTAB. Caffeine belongs to the class B, i.e. its retention decreases as the concentration of CTAB is increased both in submicellar and in micellar mobile phases. Theophylline and theobromine are class C compounds whose retention decreases as c_s is increased in submicellar mobile phases, but their retention is almost independent of the concentration of CTAB micelles. The elution order is the same in the three mobile phases, but the selectivity of separation differs from one mobile phase to another. The selectivity of separation of theophylline and theobromine is better in CTAB containing mobile phases than in aqueous methanol (Fig. 10A). The resolution of theophylline and caffeine is improved in submicellar (Fig. 10C) with respect to micellar (Fig. 10B) mobile phases, as the retention of caffeine is increased more significantly than that of the other two compounds by the shift from micellar to submicellar concentrations of CTAB.

Barbiturates belong to the class B in mobile phases containing CTAB or SDS. Phenobarbital is slightly more acidic (pK_a=7.4) than other barbiturates tested (pK_a=7.8–8.0) [25]. This may probably cause stronger interactions with CTAB (or lesser exclusion from the stationary phase, compare constants f_S in Table 4). This may contribute to increased relative retention of phenobarbital in CTAB containing micellar phases (Fig. 11B) in comparison with methanol–water mobile phases, where phenobarbital is eluted as an incompletely resolved peak between allobarbital and aprobarbital (Fig. 11A). Similar but less significant shift to higher retention volumes is observed also in micellar phases with SDS (Fig. 11C). This behaviour may be caused by the benzene ring in phenobarbital causing a decrease in the interactions with micelles with respect to other barbiturates containing only alkyl substituents (lower value of the constant K_{MW}). Overall separation selectivity is best in CTAB containing micellar mobile phases; however, the time of separation is long. The elution can be accelerated using hybrid mobile phases containing CTAB and methanol in water, where the micelles are disaggregrated. In addition to decreased time of separation, the relative retention of phenobarbital with respect to other barbiturates is decreased in the hybrid mobile phases [26].

The selectivity of separation of compounds that do not have properties of weak acids or bases in aqueous-organic mobile phases also may differ from that in submicellar mobile phases. Fig. 12 compares the separation of three derivatives of acetonitrile occurring as reaction intermediates in production of the herbicide glyphosat on a C8 column in three mobile phases. The concentrations of methanol (10%, Fig. 12A), SDS (0.003 mol/l, Fig. 12B) and CTAB (0.0005 mol/l, Fig. 12C) were adjusted to

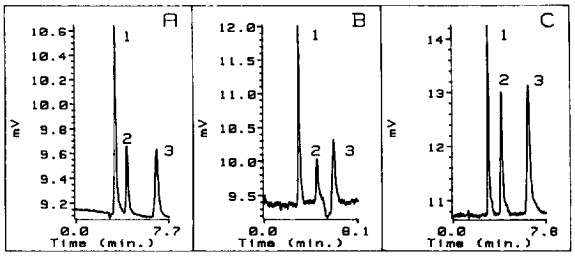

Fig. 12. Separation of derivatives of acetonitrile in aqueous-organic (A), submicellar CTAB (B) and submicellar SDS (C) mobile phases. Compounds: iminodiacetonitrile (1), nitrilotriacetonitrile (2), methylene-bis-iminodiacetonitrile (3). Column Silasorb SPH C8, 7.5 μm (300 mm × 3.6 mm I.D.); mobile phase methanol–water 10:90 (v/v) (A), 0.0005 mol/l CTAB in water (B), 0.003 mol/l SDS in water (C); flow-rate 1 ml/min, detection RI, temperature 25°C. Detector response in mV.

provide approximately equal time of separation of the sample mixture at 1 ml/min. The relative retention time of the second compound, nitrilotriacetonitrile, with respect to the other two compounds, iminodiacetonitrile and methylene-bis-iminodiacetonitrile, is shifted to provide more regular peak spacing in the chromatogram when methanol is substituted by SDS or by CTAB, the later mobile phase providing better resolution and possibilities for further optimization of the separation to decrease the time of analysis.

Separation of lower aliphatic alcohols was selected as the last example to illustrate the selectivity effects in submicellar mobile phases. Although this type of separation is typical for gas chromatography, reversed-phase HPLC with refractometric detection can be also used for this purpose. The separation of *n*-alcohols can be easily accomplished in all types of mobile phases studied, but the separation in micellar mobile phases is not practical as it requires highly concentrated alcohol samples to enable the refractometric detection, as there is very small difference between the refractive indices of the micelles and of the associates of the alcohols with the micelles. This is illustrated by the chromatogram of 20 μl of a concentrated mixture of alcohols in Fig. 13, where low responses and high baseline noise are apparent.

Fig. 14 shows remarkable differences between the separation selectivities of isomeric aliphatic alcohols

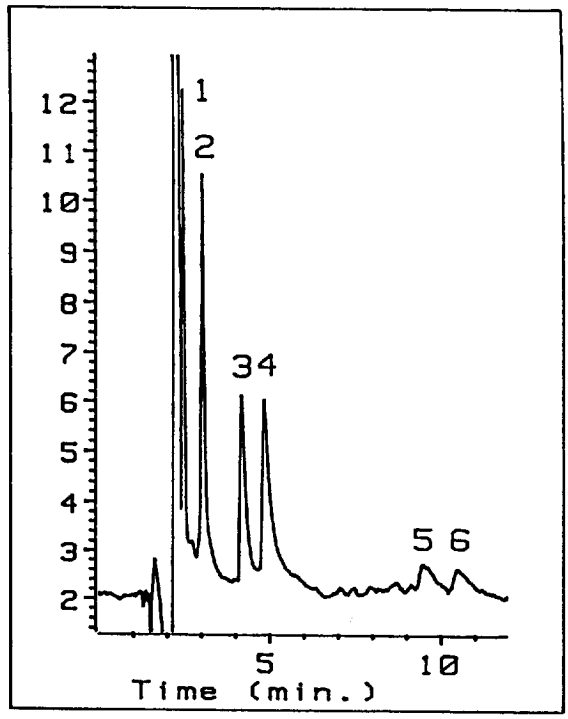

Fig. 13. Separation of alcohols in micellar mobile phase containing 30 mmol/l SDS in water. Compounds: methanol (1), ethanol (2), *iso*-propanol (3), *n*-propanol (4), *iso*-butanol (5), *n*-butanol (6). Column Silasorb SPH C8, 7.5 μm (300 mm × 3.6 mm I.D.); flow-rate 1 ml/min, detection RI, temperature 25°C. Detector response in mV.

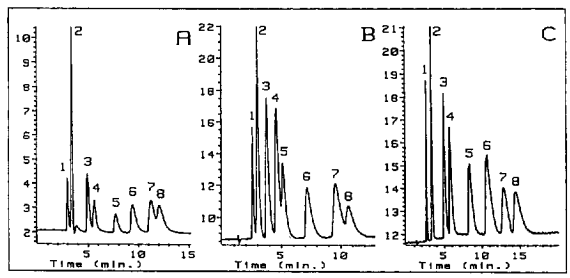

Fig. 14. Separation of alcohols in aqueous-organic (A), submicellar CTAB (B) and submicellar SDS (C) mobile phases. Compounds: methanol (1), ethanol (2), *iso*-PrOH (3), *n*-PrOH (4), *tert.*-BuOH (5), *sec.*-BuOH (6), *iso*-BuOH (7), *n*-BuOH (8). Column Silasorb SPH C8, 7.5 μm (300 mm × 3.6 mm I.D.); mobile phase acetonitrile–water 5:95 (v/v) (A), 0.0005 mol/l CTAB in water (B), 0.003 mol/l SDS in water (C); flow-rate 1 ml/min, detection RI, temperature 25°C. Detector response in mV.

in 5% aqueous acetonitrile and in submicellar mobile phases containing CTAB and SDS. In aqueous acetonitrile, the resolution between *iso*-propanol and *n*-propanol is incomplete and even worse is the resolution between *iso*-butanol and *n*-butanol (Fig. 14A). The resolution between the former pair of compounds is improved at a cost of impaired separation of *n*-propanol and *tert.*-butanol in 0.0005 mol/l CTAB, but the resolution of two last eluted butanol isomers is still poor (Fig. 14B). In 0.003 mol/l SDS, the separation selectivity is significantly improved so that it is possible to accomplish baseline separation of all isomeric C1–C4 alcohols in approximately 15 min (Fig. 14C).

5. Conclusions

Surfactants as the mobile phase additives in reversed-phase chromatography affect the retention of non-ionic analytes not only in the micellar chromatography, but also if present in sub-micellar concentrations.

The surfactant can influence the retention in different ways, depending on the nature of its interactions with the analyte. The surfactant is adsorbed on the alkylsilica bonded phase and the adsorption in submicellar solutions is controlled by the Langmuir isotherm. However, an increase in the concentration of surfactant in the micellar mobile phase does not affect the adsorbed amount which remains constant since the critical micelle concentration has been achieved. The Langmuir isotherm can be modified to account for this behaviour.

The analyte can either form associates with the adsorbed surfactant, with the surfactant in the bulk liquid or with the micelles, or it can be repulsed from the stationary phase or from the micelles. Depending on these possibilities, various non-ionic analytes can be classified into four different classes, which show different behaviour as the analytical concentration of the surfactant is increased in the submicellar and in the micellar range of mobile phase compositions: (A) the retention increases in submicellar and decreases in micellar mobile phases, (B) the retention decreases both in submicellar and micellar mobile phases, (C) the retention decreases in submicellar and is not affected in micellar mobile phases and (D) the retention decreases in submicellar and increases in micellar mobile phases.

The retention in submicellar and in micellar mobile phases can be described by simple two-parameter equations, regardless of the class of the analyte. However, the physical meaning of the constants of the retention equations in the two concentration ranges depends on the type of the interactions of the analyte with the surfactant in stationary and bulk liquid phases and with the micelles. Using experimental parameters of the adsorption isotherm and the capacity factor in pure water, the constants characterizing the distribution (or exclusion) of the analyte between the individual phases can be determined from the parameters of the retention equations. The values of the constants applying simultaneously in submicellar and micellar mobile phases determined from the independent data sets in the two concentration ranges were close to each other, which supports the underlying unified treatment of the behaviour in the submicellar and micellar range of surfactant concentrations.

The unified approach to micellar and submicellar chromatography makes it possible to estimate possible differences in selectivities of separation in the two concentration ranges, especially if the sample solutes show different dependencies of retention on the analytical concentration of the surfactant in the mobile phase. In addition to micellar chromatography or to chromatography in pure aqueous-organic mobile phases, possibly on more polar bonded stationary phases, like the cyanopropyl one, chromatography in submicellar mobile phases offers another possibility for reversed-phase separations of polar analytes requiring a low solvent strength. Various approaches may offer different selectivities and it is difficult to tell, a priori, which method is to be preferred in a particular separation problem. In addition, there are several practical advantages of submicellar chromatography with respect to the separation in micellar systems because of lower concentrations of the surfactants used in the mobile phases, namely better manipulation of retention by adjusting the concentration of the surfactant in the mobile phase, improved efficiency of separation, decreased cost of the mobile phases and better

sensitivity of refractometric detection for analytes that do not absorb light in the UV region.

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