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Journal of Chromatography B, 681 (1996) 3–19

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

# Chromatographic behaviour in reversed-phase high-performance liquid chromatography with micellar and submicellar mobile phases: effects of the organic modifier

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**Abstract**

Continuing our earlier study of the retention behaviour in reversed-phase systems with aqueous mobile phases containing surfactants in concentrations lower (submicellar systems) and higher (micellar systems) than the critical micellar concentration (CMC), we investigated the chromatographic behaviour of various non-ionic solutes in mixed aqueous–organic micellar and submicellar mobile phases and their dependence on the methanol concentration. CMC values were measured for two cationic surfactant and one anionic surfactant in mixed aqueous–methanolic solvents, and were found to increase slightly with increasing methanol concentration. Depending on the character of the surfactant, a limiting concentration of methanol was found, above which micelles do not occur anymore. Sorption isotherms of the surfactants on an octylsilica gel column were measured as a function of the concentration of methanol in aqueous–methanolic solvents. A modified Langmuir equation was used to describe the distribution of the surfactants between the stationary and the mobile phases in the concentration range below CMC. The retention of several polar solutes was measured on an octylsilica gel column both in micellar and submicellar mobile phases containing methanol. The dependencies of the capacity factors of the solutes studied on the concentration of methanol in the mobile phase can be suitably described by the same form of equation as that conventionally used for aqueous–organic mobile phases that do not contain surfactants, but the slopes of the dependencies for a given solute are different in the two ranges of surfactant concentrations. The ratio of the two slopes is controlled by the interaction with micelles and is approximately equal to, below or above 1, depending on whether the solutes do or do not associate with the micelles, or are repulsed from them. Simultaneous control of the concentrations of the organic solvent and of the surfactant in the mobile phase can be used for fine tuning the selectivity of separation as a complement to commonly used adjusting concentrations of two organic solvents in ternary aqueous–organic mobile phases. These effects are illustrated by practical examples of submicellar HPLC with mobile phases containing methanol.

*Keywords:* Mobile phase composition; Organic modifier; Critical micellar concentration

## 1. Introduction

Surfactants (surface active agents) are often used as mobile phase additives in liquid chromatography. When they are present in concentrations higher than the so-called critical micellar concentration (CMC), several surfactant molecules form aggregates — micelles. The liquid phase is then comprised of the bulk aqueous phase and of the micellar pseudo-phase. In reversed-phase chromatography, significant amounts of surfactant become adsorbed to the surface of the nonpolar stationary pnase (usually of the

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0378-4347/96/$15.00 
*SSDI* 0378-4347(95)00538-2

bonded alkylsilica gel type). Solutes are distributed between the aqueous, the micellar and the stationary phases, and the differences in the distribution of the individual sample compounds are used as the basis of the separation in micellar liquid chromatography (pseudo-phase liquid chromatography).

Even if the mobile phase contains a surfactant in concentrations lower than CMC (submicellar mobile phase), the retention of noncharged neutral compounds was found to decrease as the concentration of the adsorbed surfactant on the alkylsilica stationary phase is increased [1]. 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 ($c$) increases.

The influence of the organic solvent on the retention is similar to systems without a surfactant [2–6]. If a polar organic solvent, such as methanol or acetonitrile, is added to micellar aqueous mobile phases, the retention of analytes is decreased, which can be attributed mainly to reduced amount of the surfactant adsorbed on the surface of the nonpolar chemically-bonded column-packing material and to a decrease in the polarity of the bulk mobile phase. Further, the presence of the organic solvent may also affect the distribution of the surfactant between the micellar and the bulk liquid mobile phases.

In the low concentration range of the organic solvent the dependencies of the capacity factors, $k$, of the analytes on the concentration of the organic solvent in micellar mobile phases can be described in a similar way as analogous dependencies in the absence of the surfactant [4,5]:

$$\log k = a_m - m_{hyb}\varphi \quad (1)$$

where the parameter $a_m$ is the logarithm of the capacity factor in aqueous micellar mobile phase without the organic solvent, the slope parameter $m_{hyb}$ can be taken as the solvent strength parameter of the organic solvent in the hybrid aqueous–organic micellar mobile phase and $\varphi$ is the volume fraction of the organic solvent in the mobile phase.

As the concentration of the organic solvent in the mobile phase is gradually increased, a certain composition of the mobile phase is reached where the micelles disaggregate. In mobile phases with higher contents of the organic solvent no micelles are present. At this concentration of the organic solvent in the mobile phase transition occurs between the micellar and the submicellar mobile phases.

In submicellar mobile phases the dependence of the capacity factor of the analyte on the concentration of the organic solvent is subject to the same factors as in mobile phases without a surfactant. Consequently, the retention equation introduced earlier for aqueous–organic reversed-phase HPLC [7–9] can be expected to apply:

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

The parameters of this equation have similar meaning as the analogous parameters of Eq. 1; $a$ is the logarithm of $k$ in the mobile phase without the organic solvent and $m$ can be taken as the solvent strength parameter of the organic solvent in submicellar systems.

Adsorption of various solutes in reversed-phase systems is most often described by the Langmuir isotherm (Eq. 3) or by equations derived from the models derived from it [10]:

$$Q = \frac{ac}{1 + bc} \quad (3)$$

Here, $Q$ is the concentration of a solute in the stationary and $c$ 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 by $Q_s = a/b$. According to the Langmuir isotherm, the saturation capacity would be achieved at an infinite concentration of the solute 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 as the concentration of the surfactant in bulk solution reaches the critical micellar concentration (CMC) and the adsorbed amount remains approximately constant at concentrations higher than CMC [11–13]. To cover the break in the dependence of the adsorbed amount on $c$ at CMC, we modified the Langmuir isotherm by introducing the maximum adsorbed concentration corresponding to $c$=CMC into Eq. 3 [14]:

$$Q = \frac{a'c}{1 + b'c} = \frac{a'c}{1 + \left(\frac{a'}{Q_{CMC}} - \frac{1}{CMC}\right)c} \quad (4)$$

where $a'$ and $b'$ are constants of the modified Langmuir isotherm. This equation applies only for $c<$CMC, whereas $Q=Q_{CMC}$ for $c\geq$CMC. CMC can be determined directly using an independent method and only one batch or dynamic experiment is necessary to find the distribution corresponding to the plateau of the isotherm and $Q_{CMC}$. The distribution coefficient of the surfactant at infinite dilution, $a'$, is then the only unknown quantity in Eq. 4.

Recently we have compared the retention of various compounds in reversed-phase systems containing ionic surfactants in a wide concentration range covering both micellar and submicellar mobile phases to investigate possibilities of using submicellar instead of micellar mobile phases for separations of polar compounds [14].

Analytes can either form associates with the adsorbed surfactant, with the surfactant in the bulk liquid and in the micellar pseudo-phase, or can be excluded from the stationary phase and from the micelles. Depending on these possibilities, various nonionic analytes can be classified into four different classes, according to their behaviour when the analytical concentration of the surfactant is increased in the submicellar and micellar ranges of the mobile phase: (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 [14].

The dependence of the retention in submicellar and in micellar mobile phases on the concentration of the surfactant can be described by two simple two-parameter equations for the analytes from all the four classes, the first applying in the submicellar and the other in the micellar concentration ranges. However, the physical meaning of the constants of the two retention equations depends on the type of the interactions of the analyte with the surfactant in the stationary phase, in the bulk liquid and in the micellar pseudo-phase. Using the experimental parameters of the adsorption isotherm and capacity factor in pure water, the constants characterizing the distribution of the analyte between the individual phases can be determined from the parameters of the retention equations [14].

The aim of the present work was to extend our earlier study to the systems with micellar and submicellar mobile phases containing an organic solvent and a surfactant and to investigate the effect of the organic solvent on retention and on the selectivity of separation of the analytes belonging to the four classes mentioned above.

## 2. Experimental

### 2.1. Reagents and sample compounds

Mobile phases were prepared by mixing hexadecyltrimethylammonium bromide (CTAB, 99%+, Janssen Chimica, Beerse, Belgium or Serva, Heidelberg, Germany), N-(α-carbethoxypentadecyl)trimethylammonium bromide (Septonex, analytical grade, Slovako farma, Hlohovec, Slovak Republic) or sodium dodecyl sulphate (SDS, Aldrich, Milwaukee, WI, USA), respectively, in mixed solvents comprised of double-distilled water (with $KMnO_4$ and $NaHCO_3$) and methanol (analytical grade, Lachema, Brno, Czech Republic) in the required proportions.

Sample compounds listed in Table 2 and Table 3 were obtained from Lachema in reagent grade purities and were dissolved in the mobile phase to provide adequate detector response.

### 2.2. Determination of CMC

A conductimetric method for determination of CMC described earlier [14,15] was used. An OK-104 conductivity meter (Radelkis, Budapest, Hungary) equipped with a Metrohm conductimetric cell (Metrohm, Herishau, Switzerland) was used for the determination of CMC of CTAB, Septonex and SDS. A solution of 0.0078 mol/l CTAB, 0.0060 mol/l Septonex or of 0.0516 mol/l SDS, respectively, in

aqueous methanol (0–35%, v/v) was gradually added from the burette to 50 ml of the mixed solvent of the same composition 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 a plot of the conductivity versus the concentration of the surfactant in the solution in the beaker, taking into account necessary corrections for changing volume during the "titration". The CMC was determined as the point of intersection of the straight line parts of the conductimetric "titration curves" below and above the CMC. The conductivity meter was calibrated using a standard solution of 0.001 mol/l KCl (analytical grade, Lachema).

### 2.3. Sorption isotherms

Sorption isotherms of surfactants were measured by the frontal chromatography method [16,17] using two M 6000A pumps (Waters-Millipore, Milford, MA, USA) controlled by an M 660 gradient programmer (Waters) to stepwise increase the concentration of the surfactant in methanol–water mixture. The solution of the surfactant was pumped through a short stainless steel sorption column, 24×3.9 mm I.D. (Waters), packed in the laboratory with Silasorb SPH $C_8$, 7.5 $\mu$m, spherical octylsilica material (Lachema) connected to a LCD 2040 variable-wavelength detector (ECOM, Prague, Czech Republic) set to 224 nm (for the isotherms of CTAB and Septonex) or to a CDLC 1 conductimetric detector (Laboratory Instrument Works, Prague, Czech Republic, for the isotherms of SDS). The solutions used were filtrated through 0.45-$\mu$m membrane filters (Millipore) and degassed by ultrasonication prior to the use. The columns were thermostatted at 25°C.

One pump (A) delivered a pure methanol–water mixture and another pump (B) the solution of the surfactant containing 0.004 mol/l CTAB, 0.004 mol/l Septonex or 0.015 mol/l SDS in the aqueous methanol of the same composition. The concentration of the surfactant in 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). 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 and the volume of the stationary phase was calculated as the difference between the total volume of the column and the column dead volume. A freshly packed column was employed for measuring the sorption isotherms of each surfactant.

### 2.4. Elution data

A HP 1090M liquid chromatograph (Hewlett-Packard, 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, 300×4.2 mm I.D., packed in the laboratory with Silasorb SPH $C_8$, 7.5 $\mu$m (Lachema), was used for the experiments with CTAB. A freshly packed column of the same dimensions and packing material was used for the experiments with Septonex. For the experiments with SDS, a stainless steel column, 300×3.6 mm I.D., was used. Mobile phases were prepared by dissolving the surfactant in mixtures of water (double-distilled in glass with an addition of $KMnO_4$) and methanol in appropriate proportions and were filtrated through a 0.45-$\mu$m membrane filter prior to use. The flow-rate of the mobile phases was kept at 1 ml/min in all the experiments.

The column used was first equilibrated with the mobile phase comprised of surfactant and water and then the retention volumes of all test compounds were measured. In the subsequent sets of experiments, the methanol content in the hybrid mobile phases was increased. Freshly prepared columns were employed for each of the sets of experiments

with mobile phases containing either CTAB, Septonex or SDS. To calculate the capacity factors, $k=V_R/V_0-1$, column dead volumes $V_0$ were determined after injection of $D_2O$ with refractometric detection (R 401 Differential Refractometer, Waters).

## 3. Results and discussion

### 3.1. Dependence of the critical micellar concentration on the concentration of methanol

Table 1 and Fig. 1 illustrate the dependence of the experimental values of CMC of the cationic surfactants CTAB and Septonex and of the anionic surfactant SDS on the concentration of methanol in mixed aqueous–methanolic solvents. As the concentration of the organic solvent is increased, the critical micellar concentration (CMC) also increases, which means that the distribution of the surfactant between the bulk mobile and the micellar phases is shifted towards the former one. This effect is not very dramatic until a concentration of the organic solvent is reached where the micelles disaggregate, so that the micellar mobile phase is converted to the submicellar phase. The CMC of CTAB first rises as the concentration of methanol is increased from 0 to 10% and is almost constant in solvents with 10–19% of methanol, while the micelles do not exist in solutions with more than 20% methanol. The CMC values of SDS are practically unaffected by methanol

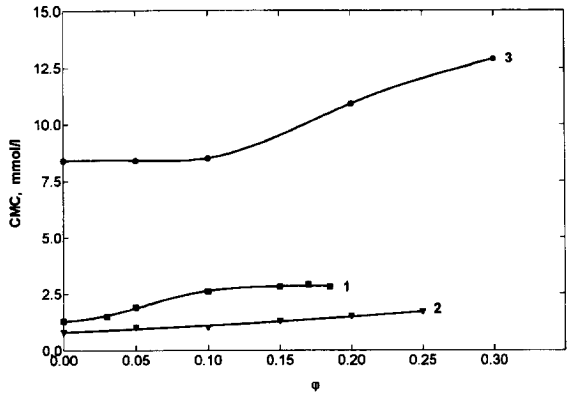


Fig. 1. Dependence of the critical micellar concentration (CMC) of CTAB (1), Septonex (2) and SDS (3) on the concentration of methanol ($\varphi$, in vol%$\times 10^{-2}$) in aqueous–methanolic solvents.

Table 1
CMC, parameters of the isotherms (Eq. 3 and Eq. 4) and concentrations of the surfactant adsorbed in the stationary phase $Q_{CMC}$ (Eq. 4) and $Q_S=a/b$ (Eq. 3) of cetyltrimethylammonium bromide (CTAB), Septonex and SDS in methanol–water solutions at 25°C

| Methanol (%) | CMC (mmol/l) | Modified Langmuir isotherm (Eq. 4) | | | Langmuir isotherm (Eq. 3) | | |
|---|---|---|---|---|---|---|---|
| | | $a'$ | $b'$ (l/mol) | $Q_{CMC}$ (mol/l) | $a$ | $b$ (l/mol) | $Q_S$ (mol/l) |
| *CTAB* | | | | | | | |
| 0 | 1.3 | 4958 | 3838 | 1.025 | 6930 | 6115 | 1.133 |
| 5 | 1.9 | 4365 | 3556 | 1.000 | 5764 | 5221 | 1.104 |
| 10 | 2.6 | 3086 | 2521 | 0.968 | 3942 | 3591 | 1.098 |
| 30 | – | | | | 436 | 312 | 1.394 |
| *Septonex* | | | | | | | |
| 0 | 0.8 | 7325 | 9174 | 0.720 | 7857 | 10 037 | 0.782 |
| 10 | 1.0 | 2973 | 5476 | 0.584 | 3409 | 5095 | 0.669 |
| 20 | 1.5 | 1156 | 588 | 0.521 | 1274 | 1928 | 0.661 |
| 30 | – | | | | 379 | 558 | 0.644 |
| *SDS* | | | | | | | |
| 0 | 8.4 | 605 | 384 | 1.205 | 684 | 465 | 1.471 |
| 0.10 | 8.5 | 440 | 338 | 0.980 | 490 | 402 | 1.219 |
| 0.20 | 10.9 | 224 | 196 | 0.756 | 280 | 291 | 0.962 |
| 0.40 | – | | | | 52.1 | 141 | 0.369 |

–, micelles are not formed.

in concentrations below 10%, but raise as the concentration of methanol is further increased, whereas only a slight increase in CMC of Septonex occurs in mobile phases with more than 10% of methanol. The micelles of Septonex and of SDS are not formed in mobile phases with more than 25 or 30% methanol, respectively.

### *3.2. Sorption isotherms of anionic and cationic surfactants*

The parameters $a$ and $b$ of the Langmuir isotherm and the parameters $a'$ and $b'$ of the modified isotherm (Eq. 4) and the values of maximum sorption capacities ($Q_S=a/b$ and $Q_{CMC}$ are listed in Table 1. Fig. 2, Fig. 3 and Fig. 4 show the experimental isotherms (points) of CTAB, Septonex and SDS on a Silasorb $C_8$ column in mobile phases with various concentrations of methanol in water. The isotherms calculated from Eq. 4 are plotted as full lines in the submicellar range of surfactant concentrations. For comparison, broken lines represent the unmodified Langmuir isotherm (Eq. 3) fitted to the experimental data. The fit of the modified isotherm (Eq. 4) in the submicellar concentration range is comparable with or better than that of the unmodified Langmuir isotherm. The experimental data for the isotherms of CTAB and Septonex in 30% methanol and of SDS in 40% methanol where the micelles do not exist are fitted by the unmodified Langmuir isotherm (Eq. 3). The adsorbed concen-

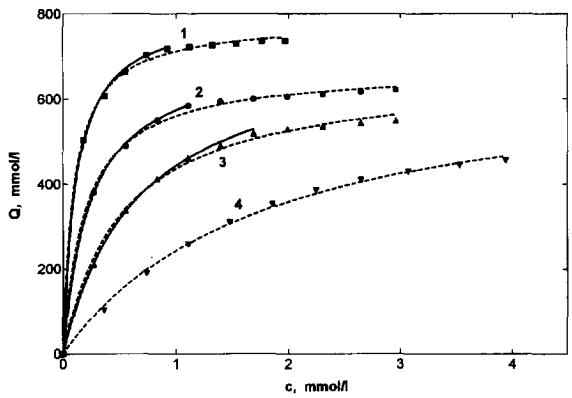


Fig. 3. Sorption isotherms of Septonex on a Silasorb SPH $C_8$, 7.5 $\mu$m, 24×3.9 mm I.D. column in aqueous–methanolic solvents: 100% water (1); 10% (v/v) methanol (2); 20% (v/v) methanol (3); 30% (v/v) methanol (4). $Q$, concentration of Septonex in the stationary phase; $c$, concentration of Septonex in the mobile phase; dots, experimental data; broken lines, Langmuir equation (Eq. 3); full lines, modified Langmuir equation (Eq. 4), submicellar range only, fitted to the experimental data.

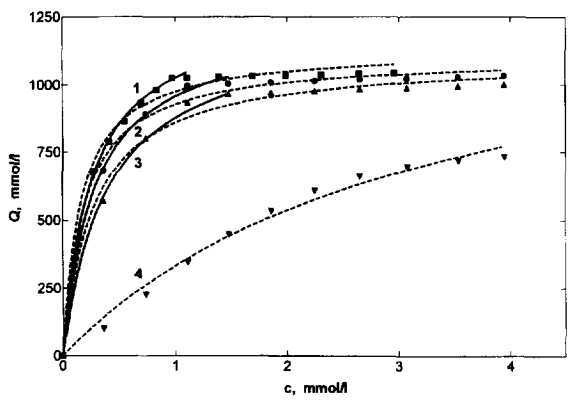


Fig. 2. Sorption isotherms of CTAB on a Silasorb SPH $C_8$, 7.5 $\mu$m, 24×3.9 mm I.D. column in aqueous–methanolic solvents: 100% water (1); 5% (v/v) methanol (2); 10% (v/v) methanol (3); 30% (v/v) methanol (4). $Q$, concentration of CTAB in the stationary phase; $c$, concentration of CTAB in the mobile phase; dots, experimental data; broken lines, Langmuir equation (Eq. 3); full lines, modified Langmuir equation (Eq. 4), submicellar range only, fitted to the experimental data.

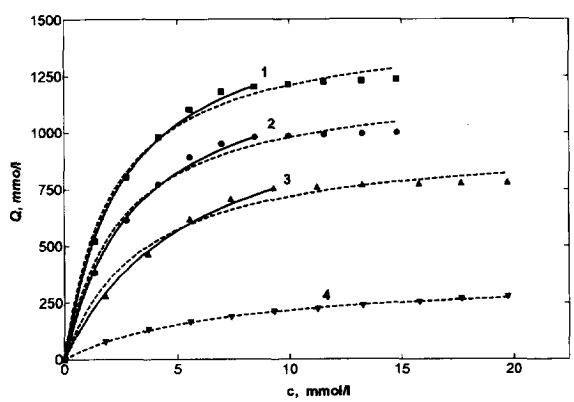


Fig. 4. Sorption isotherms of SDS on a Silasorb SPH $C_8$, 7.5 $\mu$m, 24×3.9 mm I.D. column in aqueous–methanolic solvents: .100% water (1); 10% (v/v) methanol (2); 20% (v/v) methanol (3); 40% (v/v) methanol (4). $Q$, concentration of SDS in the stationary phase; $c$, concentration of SDS in the mobile phase; dots, experimental data; broken lines, Langmuir equation (Eq. 3); full lines, modified Langmuir equation (Eq. 4), submicellar range only, fitted to the experimental data.

tration of the surfactant $Q$ decreases as the concentration of methanol in the mobile phase is raised. The rates of the decrease of the adsorbed concentrations of CTAB and Septonex are more important in submicellar than in micellar phases, but with SDS are similar in the two types of phases, as illustrated in Fig. 5. The influence of the concentration of methanol on the values of $Q$ in micellar phases is more significant with SDS than with CTAB and Septonex.

The capacity factor of the surfactant at infinite dilution, $k_s$, can be determined from the parameters $a$ ($a'$) as $k_s = a\Phi$, where $\Phi$ denotes the phase ratio, i.e., the ratio of the volumes of the stationary ($V_S = V_C - V_0$) and of the mobile ($V_0$) phases in the column with a total volume $V_C$. Fig. 6 shows the dependencies of the logarithms of $k_s$ on the concentration of methanol in the mobile phase. The plots are almost linear for Septonex and SDS and slightly curved for CTAB.

### 3.3. Effect of methanol on chromatographic behaviour in micellar and submicellar phases

To investigate the effect of methanol on the chromatographic behaviour in micellar and submicellar mobile phases, capacity factors of various analytes were measured in mobile phases containing 0.03 mol/l CTAB, Septonex and SDS and various concentrations of methanol. The data are given in Table 2, Table 3 and Table 4. The concentration range of 0–40% methanol for CTAB and 0–50% methanol for Septonex comprises both micellar and submicellar mobile phases and the transition between the two types of phases occurs in 19% methanol with CTAB and in between 25 and 30% methanol with Septonex. The data for 0.03 mol/l SDS in 0–30% methanol relate to micellar mobile phases only. The plots of the logarithms of the capacity factors of various analytes versus the concentration of methanol in the mobile phase (Fig. 7 and Fig. 8) show two different sections corresponding to the micellar and to the submicellar concentration ranges. Within each range, the plots are almost linear, but have different slopes, corresponding to different values of the parameters $a$, $m$ and $a_m$, $m_{hyb}$ of the retention Eqs. 1 and 2 for the analytes studied (Table 5, Table 6 and Table 7). Most analytes have lower intercepts, $a_m$, and the slopes, $m_{hyb}$, of the dependencies (Eq. 1) in micellar mobile phases in comparison to submicellar mobile phases ($a$ and $m$ in Eq. 2) both in systems with CTAB and with Septonex. However, the opposite behaviour was observed with pyridine, aniline, theophylline and caffeine in CTAB con-

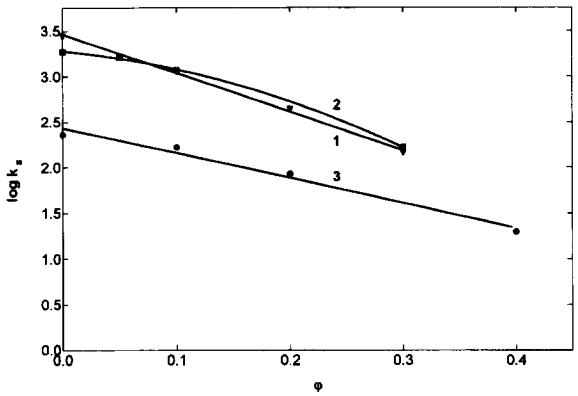


Fig. 6. Effect of the concentration of methanol ($\varphi$, vol%$\times 10^{-2}$) on the capacity factors of CTAB (1), Septonex (2) and SDS (3) at infinite dilution, $k_s$, on a Silasorb SPH $C_8$ column.

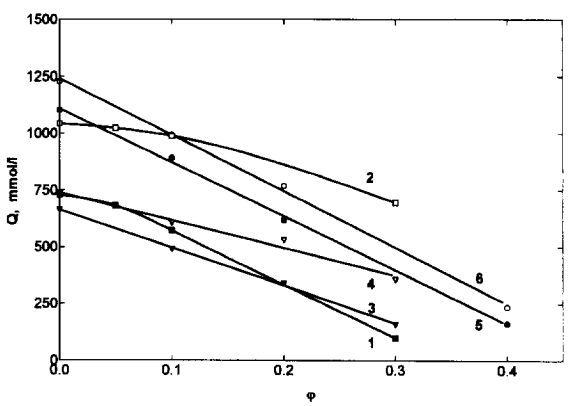


Fig. 5. Dependence of the concentrations of surfactants ($Q$, mmol/l) adsorbed to a Silasorb SPH $C_8$ column on the concentration of the methanol content ($\varphi$, vol%$\times 10^{-2}$) in micellar and submicellar mobile phases containing 0.36 mmol/l CTAB (1), 3.0 mmol/l CTAB (2), 0.55 mmol/l Septonex (3), 1.98 mmol/l Septonex (4), 5.55 mmol/l SDS (5) and 13.25 mmol/l SDS (6). Full markers correspond to the data points in submicellar and empty markers to those in micellar range of mobile phase composition.

Table 2
Capacity factor $k$ of polar compounds on a Silasorb SPH $C_8$, 7.5 μm, 300×3.6 mm I.D. column in mobile phases containing 0.03 mol/l CTAB and $\varphi \times 10^{-2}$% methanol at 25°C

| Compound | $k$ at $\varphi_{MeOH}$ | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
| Barbital | 1.72 | 1.56 | 1.44 | 1.08 | 0.77 | 0.53 |
| Allobarbital | 4.67 | 4.13 | 3.68 | 2.81 | 1.84 | 1.06 |
| Aprobarbital | 6.19 | 5.70 | 5.09 | 4.24 | 2.51 | 1.54 |
| Butobarbital | 8.87 | 8.13 | 7.19 | 5.50 | 3.70 | 2.05 |
| Phenobarbital | 10.7 | 10.1 | 9.18 | 7.31 | 4.57 | 2.47 |
| Pentobarbital | 14.4 | 13.3 | 12.0 | 9.42 | 6.37 | 3.72 |
| Hexobarbital | 19.1 | 16.2 | 13.3 | 9.05 | 5.69 | 3.39 |
| Theophylline | 2.06 | 1.55 | 1.05 | 0.63 | 0.34 | 0.23 |
| Caffeine | 0.97 | 0.75 | 0.61 | 0.38 | 0.28 | 0.20 |
| Phenol | 24.0 | 20.0 | 17.1 | 10.9 | 6.68 | 3.94 |
| Pyrocatechol | 25.6 | 19.5 | 14.8 | 9.49 | 6.30 | 3.40 |
| Resorcinol | 17.5 | 14.2 | 12.1 | 7.25 | 4.26 | 1.96 |
| Hydroquinone | 7.41 | 5.82 | 4.84 | 3.17 | 1.92 | 1.09 |
| Phloroglucinol | 5.13 | 4.70 | 4.19 | 2.58 | 1.57 | 0.82 |
| *m*-Cresol | 35.5 | 30.5 | 27.0 | 19.0 | 13.0 | 8.81 |
| Acetophenone | 14.6 | 12.4 | 10.5 | 7.22 | 4.52 | 2.64 |
| Anisole | 22.1 | 20.5 | 18.7 | 14.0 | 10.3 | 5.86 |
| Benzene | 21.8 | 18.5 | 17.0 | 13.3 | 9.33 | 6.25 |
| Benzonitrile | 13.8 | 11.9 | 10.2 | 7.10 | 4.61 | 2.61 |
| Nitrobenzene | 20.9 | 17.3 | 15.8 | 10.9 | 7.74 | 5.12 |
| *m*-Bromonitrobenzene | 38.0 | 34.0 | 31.0 | 23.9 | 16.8 | 11.2 |
| Aniline | 12.9 | 9.14 | 6.69 | 4.49 | 3.04 | 1.96 |
| Pyridine | 4.21 | 2.51 | 1.50 | 1.12 | 0.91 | 0.62 |

taining mobile phases and with pyridine in mobile phases containing Septonex. These compounds belong to the classes C and D, according to the earlier observed behaviour in aqueous micellar and submicellar mobile phases containing CTAB [14]. A lower increase in the $m_{hyb}$ with respect to the $m$ values was observed for compounds that do not associate with micelles (theophylline and caffeine, class C) than for the basic compounds (pyridine, aniline) that are excluded from the micelles (class D). This behaviour is apparent to a lesser extent in mobile phases with Septonex, where only the $m_{hyb}$ of pyridine is higher than $m$, whereas $m_{hyb}$ is slightly lower than $m$ with caffeine (with other compounds the decrease in $m_{hyb}$ with respect to $m$ is much more significant).

A possible explanation may take into account the equilibrium between the free analytes in the bulk mobile phase and in the micellar pseudo-phase. If the analytes are associated with the micelles and no important interactions occur with the surfactant adsorbed in the stationary phase, the retention (capacity factor $k$) can be described by Eq. 5 [14]:

$$k = k_{CMC} \frac{1}{1 + K_{MW} c_m} \quad (5)$$

where $k_{CMC}$ is the capacity factor in the mobile phase where the concentration of the surfactant is equal to CMC and the concentration of micelles $c_m = 0$, and $K_{MW}$ is the equilibrium constant describing the distribution of the analyte between the micellar pseudo-phase and the bulk mobile phase. In the absence of the micelles, methanol controls the retention only by means of the effect on $k_{CMC}$. As the concentration of methanol in the mobile phase increases, CMC increases and the concentration of the micelles, $c_m$, decreases (Fig. 1), which results in decreasing equilibrium concentration of the associates of the analytes with the micelles. Decreasing

Table 3
Capacity factor $k$ of polar compounds on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in mobile phases containing 0.03 mol/l Septonex and $\varphi\times10^{-2}$% methanol at 25°C

| Compound | $k$ at $\varphi_{MeOH}$ | | | | | |
|---|---|---|---|---|---|---|
| | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 |
| Barbital | 1.06 | 0.97 | 0.89 | 0.67 | 0.45 | 0.28 |
| Allobarbital | 2.70 | 2.44 | 1.98 | 1.51 | 0.89 | 0.53 |
| Aprobarbital | 3.54 | 3.27 | 2.62 | 1.98 | 1.18 | 0.71 |
| Phenobarbital | 7.02 | 6.14 | 4.68 | 2.90 | 1.89 | 1.05 |
| Pentobarbital | 9.19 | 8.53 | 7.11 | 5.31 | 3.07 | 1.46 |
| Hexobarbital | 8.26 | 7.49 | 5.93 | 4.10 | 2.45 | 1.32 |
| Theophylline | 0.65 | 0.54 | 0.45 | 0.32 | 0.19 | 0.12 |
| Caffeine | 0.79 | 0.64 | 0.48 | 0.36 | 0.23 | 0.17 |
| Phenol | 11.2 | 10.4 | 7.9 | 4.94 | 3.02 | 1.93 |
| Pyrocatechol | 9.35 | 8.30 | 6.41 | 3.77 | 2.52 | 1.63 |
| Resorcinol | 7.85 | 6.38 | 4.67 | 2.68 | 1.44 | 0.65 |
| Hydroquinone | 2.80 | 2.41 | 1.90 | 1.12 | 0.64 | 0.37 |
| Phloroglucinol | 2.92 | 2.36 | 1.94 | 1.14 | 0.67 | 0.38 |
| *m*-Cresol | – | – | 13.6 | 8.85 | 5.30 | 3.78 |
| Acetophenone | 7.65 | 6.93 | 5.32 | 2.18 | 1.35 | 0.97 |
| Anisole | 15.5 | 14.0 | 12.0 | 8.37 | 5.34 | 3.58 |
| Benzene | 14.1 | 13.3 | 11.7 | 8.50 | 5.68 | 3.12 |
| Toluene | – | – | 20.7 | 15.4 | 10.9 | 6.95 |
| Benzonitrile | 7.31 | 6.66 | 5.28 | 3.66 | 2.12 | 1.31 |
| Nitrobenzene | 11.9 | 10.7 | 8.90 | 6.48 | 4.07 | 2.07 |
| *m*-Bromonitrobenzene | – | – | 20.0 | 15.1 | 10.3 | 6.76 |
| Aniline | 4.15 | 3.79 | 3.32 | 2.18 | 1.35 | 0.97 |
| Pyridine | 2.32 | 1.61 | 0.84 | 0.78 | 0.57 | 0.48 |

the product $K_{MW}\cdot c_m$ in the denominator of Eq. 5 as the concentration of methanol is increased results in a less steep decrease of $k$ in micellar than in

Table 4
Capacity factor $k$ of polar compounds on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in mobile phases containing 0.03 mol/l SDS and $\varphi\times10^{-2}$% methanol at 25°C

| Compound | $k$ at $\varphi_{MeOH}$ | | | | |
|---|---|---|---|---|---|
| | 0 | 0.05 | 0.10 | 0.20 | 0.30 |
| Barbital | 4.19 | 3.21 | 2.54 | 1.51 | 0.85 |
| Allobarbital | 10.2 | 7.91 | 6.60 | 3.70 | 2.21 |
| Aprobarbital | 16.9 | 13.5 | 10.6 | 5.94 | 3.41 |
| Phenobarbital | 17.2 | 14.0 | 11.0 | 5.89 | 3.30 |
| Theophylline | 1.55 | 1.10 | 0.71 | 0.43 | 0.25 |
| Caffeine | 3.50 | 2.16 | 1.45 | 0.72 | 0.44 |
| Resorcinol | 1.33 | 1.09 | 0.84 | 0.56 | 0.36 |
| Hydroquinone | 0.43 | 0.32 | 0.24 | 0.15 | 0.11 |
| Acetophenone | 23.2 | 18.1 | 13.6 | 7.95 | 4.87 |
| Benzonitrile | 16.4 | 13.1 | 10.0 | 6.38 | 4.02 |

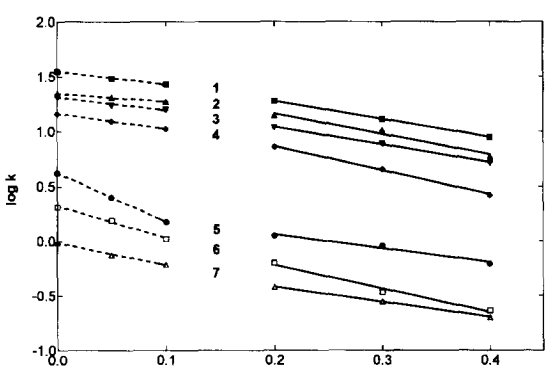


Fig. 7. Dependence of the capacity factors ($k$) of *m*-cresol (1), anisole (2), nitrobenzene (3), acetophenone (4), pyridine (5), theophylline (6) and caffeine (7) on the concentration of methanol ($\varphi$, vol%×$10^{-2}$) in aqueous–methanolic mobile phases containing 0.03 mol/l CTAB. Full lines, micellar mobile phases; broken lines, submicellar mobile phases.

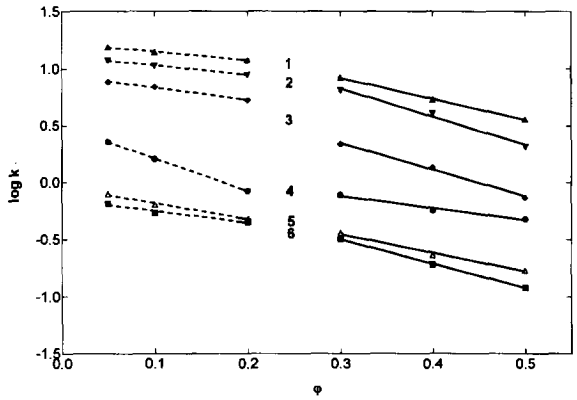


Fig. 8. Dependence of the capacity factors ($k$) of anisole (1), nitrobenzene (2), acetophenone (3), pyridine (4), caffeine (5) and theophylline (6) on the concentration of methanol ($\varphi$, vol%$\times 10^{-2}$) in aqueous–methanolic mobile phases containing 0.03 mol/l Septonex. Full lines, micellar mobile phases; broken lines, submicellar mobile phases.

submicellar systems and consequently in lower $m_{hyb}$ values with respect to $m$ (this effect can lead to non-linearity of the $\log k$ versus $\varphi$ plots).

If the analytes are neither associated with nor excluded by the molecules of the surfactant adsorbed in the stationary phase, but are excluded from the micelles, the retention equation acquires the following form [14]:

$$k = k_{CMC} \cdot \frac{1}{1 - f_{mic} \cdot c_m} \quad (6)$$

Here, $f_{mic}$ is a proportionality constant characterizing the part of the column void volume that is inaccessible to the analyte because of the exclusion from the micelles [14]. As the concentration of methanol increases in the mobile phase, the concentration of the micelles and the product $f_{mic} \cdot c_m$ decrease, so that

Table 5
Parameters $a$ and $m$ of Eq. 2 in submicellar mobile phases (0–10% methanol in water) and parameters $a_m$ and $m_{hyb}$ of Eq. 1 in micellar mobile phases (20–40% methanol in water) containing 0.03 mol/l CTAB for the analytes from Table 2 on a Silasorb SPH $C_8$ column

| Compound | Eq. 1 | | | Eq. 2 | | | Ratio $m_{hyb}/m$ |
|---|---|---|---|---|---|---|---|
| | $a_m$ | $m_{hyb}$ | $r$ | $a$ | $m$ | $r$ | |
| Barbital | 0.234 | 0.772 | 0.998 | 0.345 | 1.546 | 0.999 | 0.50 |
| Allobarbital | 0.668 | 1.035 | 0.999 | 0.881 | 2.117 | 0.997 | 0.48 |
| Aprobarbital | 0.794 | 0.849 | 0.996 | 1.064 | 2.199 | 0.999 | 0.39 |
| Butobarbital | 0.950 | 0.912 | 0.995 | 1.183 | 2.143 | 0.994 | 0.43 |
| Phenobarbital | 1.031 | 0.661 | 0.991 | 1.345 | 2.356 | 0.997 | 0.28 |
| Pentobarbital | 1.160 | 0.783 | 0.997 | 1.388 | 2.018 | 0.995 | 0.39 |
| Hexobarbital | 1.282 | 1.554 | 0.999 | 1.387 | 2.132 | 0.999 | 0.73 |
| Theophylline | 0.301 | 2.927 | 0.989 | 0.221 | 2.188 | 0.991 | 1.33 |
| Caffeine | −0.017 | 2.014 | 0.998 | −0.139 | 1.394 | 0.999 | 1.44 |
| Phenol | 1.378 | 1.474 | 0.999 | 1.458 | 2.217 | 0.999 | 0.66 |
| Pyrocatechol | 1.411 | 2.411 | 0.999 | 1.438 | 2.228 | 0.993 | 1.08 |
| Resorcinol | 1.239 | 1.569 | 0.997 | 1.446 | 2.840 | 0.994 | 0.56 |
| Hydroquinone | 0.865 | 1.849 | 0.997 | 0.965 | 2.318 | 0.999 | 0.80 |
| Phloroglucinol | 0.712 | 0.879 | 0.997 | 0.920 | 2.489 | 0.997 | 0.35 |
| *m*-Cresol | 1.545 | 1.165 | 0.999 | 1.611 | 1.663 | 0.999 | 0.70 |
| Acetophenone | 1.164 | 1.425 | 0.999 | 1.300 | 2.185 | 0.999 | 0.65 |
| Anisole | 1.346 | 0.729 | 0.998 | 1.524 | 1.888 | 0.986 | 0.40 |
| Benzene | 1.322 | 0.936 | 0.992 | 1.543 | 1.633 | 0.999 | 0.59 |
| Benzonitrile | 1.140 | 1.299 | 0.999 | 1.296 | 2.173 | 0.996 | 0.60 |
| Nitrobenzene | 1.304 | 1.260 | 0.997 | 1.372 | 1.644 | 0.999 | 0.77 |
| *m*-Bromonitrobenzene | 1.578 | 0.892 | 0.999 | 1.708 | 1.635 | 0.999 | 0.55 |
| Aniline | 1.110 | 2.862 | 0.999 | 1.015 | 1.800 | 0.999 | 1.59 |
| Pyridine | 0.624 | 4.481 | 0.999 | 0.319 | 1.284 | 0.986 | 3.50 |

$r$, correlation coefficient.

Table 6
Parameters $a$ and $m$ of Eq. 2 in submicellar mobile phases (0–10% methanol in water) and parameters $a_m$ and $m_{hyb}$ of Eq. 1 in micellar mobile phases (20–40% methanol in water) containing 0.03 mol/l Septonex for the analytes from Table 3 on a Silasorb SPH $C_8$ column

| Compound | Eq. 1 | | | Eq. 2 | | | Ratio $m_{hyb}/m$ |
|---|---|---|---|---|---|---|---|
| | $a_m$ | $m_{hyb}$ | $r$ | $a$ | $m$ | $r$ | |
| Barbital | 0.043 | 0.487 | 0.980 | 0.400 | 1.849 | 0.998 | 0.26 |
| Allobarbital | 0.477 | 0.900 | 0.999 | 0.860 | 2.273 | 0.999 | 0.40 |
| Aprobarbital | 0.597 | 0.884 | 0.997 | 0.962 | 2.227 | 0.999 | 0.40 |
| Phenobarbital | 0.905 | 1.175 | 0.999 | 1.136 | 2.206 | 0.999 | 0.53 |
| Pentobarbital | 1.003 | 0.749 | 0.999 | 1.580 | 2.803 | 0.996 | 0.27 |
| Hexobarbital | 0.968 | 0.967 | 0.999 | 1.358 | 2.461 | 0.999 | 0.39 |
| Theophylline | −0.147 | 1.026 | 0.981 | 0.139 | 2.129 | 0.993 | 0.48 |
| Caffeine | −0.040 | 1.415 | 0.994 | 0.034 | 1.629 | 0.994 | 0.87 |
| Phenol | 1.104 | 1.039 | 0.990 | 1.303 | 2.040 | 0.996 | 0.51 |
| Pyrocatechol | 1.027 | 0.097 | 0.999 | 1.125 | 1.820 | 0.999 | 0.60 |
| Resorcinol | 0.962 | 1.438 | 0.997 | 1.364 | 3.076 | 0.997 | 0.48 |
| Hydroquinone | 0.498 | 1.110 | 0.998 | 0.757 | 2.347 | 0.999 | 0.47 |
| Phloroglucinol | 0.508 | 1.136 | 0.977 | 0.775 | 2.386 | 0.999 | 0.48 |
| *m*-Cresol | – | – | – | 1.488 | 1.847 | 0.993 | – |
| Acetophenone | 0.941 | 1.065 | 0.997 | 1.127 | 1.968 | 0.998 | 0.54 |
| Anisole | 1.224 | 0.727 | 0.997 | 1.472 | 1.844 | 0.999 | 0.39 |
| Benzene | 1.178 | 0.549 | 0.999 | 1.594 | 2.169 | 0.994 | 0.25 |
| Toluene | – | – | – | 1.705 | 1.728 | 0.999 | – |
| Benzonitrile | 0.914 | 0.951 | 0.999 | 1.228 | 2.231 | 0.999 | 0.43 |
| Nitrobenzene | 1.114 | 0.829 | 0.999 | 1.570 | 2.478 | 0.994 | 0.33 |
| *m*-Bromonitrobenzene | – | – | – | 1.704 | 1.743 | 0.999 | – |
| Aniline | 0.647 | 0.636 | 0.997 | 0.855 | 1.758 | 0.994 | 0.36 |
| Pyridine | 0.506 | 2.923 | 0.999 | 0.198 | 1.054 | 0.986 | 2.75 |

$r$, correlation coefficient.

the denominator in Eq. 6 increases, which causes a steeper decrease of $k$ in micellar than in submicellar systems.

Table 7
Parameters $a_m$ and $m_{hyb}$ of Eq. 1 in micellar mobile phases containing 0.03 mol/l SDS in 0–30% methanol in water for the analytes from Table 4 on a Silasorb SPH $C_8$ column

| Compound | Eq. 1 | | |
|---|---|---|---|
| | $a_m$ | $m_{hyb}$ | $r$ |
| Barbital | 0.623 | 2.777 | 0.999 |
| Allobarbital | 1.018 | 2.232 | 0.999 |
| Aprobarbital | 1.242 | 2.344 | 0.999 |
| Phenobarbital | 1.259 | 2.438 | 0.998 |
| Theophylline | 0.160 | 2.595 | 0.995 |
| Caffeine | 0.495 | 2.979 | 0.993 |
| Resorcinol | 0.139 | 1.962 | 0.996 |
| Hydroquinone | −0.394 | 1.958 | 0.992 |
| Acetophenone | 1.364 | 2.278 | 0.999 |
| Benzonitrile | 1.216 | 2.043 | 0.999 |

$r$, correlation coefficient.

This explanation is only a qualitative one and does not take full account of all possible effects of methanol in the mobile phase such as a steeper decrease of the adsorbed amount of the surfactant in submicellar than in micellar mobile phases as the concentration of methanol is increased (Fig. 5).

The constants $a$, $a_m$ (the logarithms of $k$ in submicellar and micellar phases, respectively, in the absence of methanol) and $m_{hyb}$ in Eqs. 1 and 2 are higher for most analytes tested in the systems with CTAB than in those containing Septonex, while the differences between the constants $m$ relating to submicellar phases with CTAB and with Septonex are much less significant. This can possibly be attributed to larger amounts of CTAB adsorbed to the stationary phase in comparison with Septonex and to increased possibility of interactions of polar analytes with the ethylester group in the molecules of Septonex. The relative decrease of $m_{hyb}$ with respect to $m$ is more significant in the systems with Septonex

than in those containing CTAB for most compounds tested and the ratio $m_{hyb}/m$ can also be affected by the possible formation of complexes with the surfactants adsorbed to the stationary phase (such as phenolic compounds with the surfactants of the quaternary amine type) or by exclusion of some analytes from the micelles and from the stationary phase (e.g., basic compounds when cationic surfactants are used [14]).

### *3.4. Effects of methanol on the separation selectivity*

The results of the present study suggest that the concentration of the organic solvent can be varied in addition to adjusting the concentration of the surfactant in the mobile phase to provide an efficient tool for controlling the retention and the separation selectivity in micellar and in submicellar systems. Further, addition of an organic solvent to the mobile phase may improve the solubility of some analytes. Finally, simultaneous control of the concentrations of the surfactant and of the organic solvent has an effect similar to adjusting the composition of a ternary mobile phase comprised of water and two organic solvents, and offers another possibility for fine-tuning of the selectivity and for optimization of separation.

For example, Fig. 9 illustrates significant selectivity differences for five polyhydroxybenzenes on a $C_8$ column in 30% methanol and in submicellar and micellar mobile phases containing SDS or CTAB in aqueous methanol. Because of the neutral pH, the phenols were not dissociated so that no real ion-pairing interactions were effective. The resolution of the sample compounds was much better in 30% methanol (Fig. 9A) than in submicellar mobile phase containing 0.03 mol/l CTAB in 30% methanol (Fig. 9B). Submicellar aqueous solution of 0.003 mol/l SDS shows significantly higher selectivity of separation of dihydroxybenzenes with respect to the aqueous methanol and suppression of the peak tailing (Fig. 9C). The separation factor of resorcinol vs. hydroquinone increased from 2.0 to 2.8 and that of pyrocatechol vs. resorcinol from 1.5 to 2.7. In hybrid mobile phases containing 0.03 mol/l SDS in 10% methanol (Fig. 9D) the selectivity was similar, but

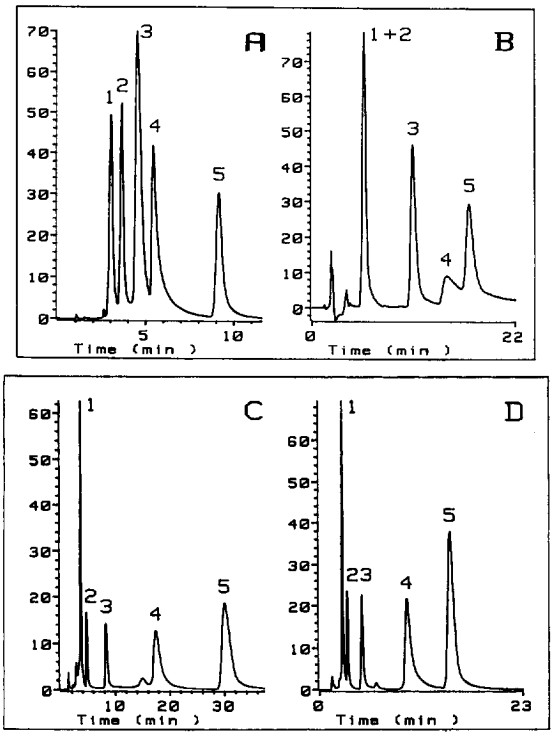


Fig. 9. Separation of a mixture of phenols on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. in 30% methanol in water (A) and in a submicellar mobile phase containing 0.03 mol/l CTAB in 30% methanol (B), in a submicellar mobile phase containing 0.003 mol/l SDS in water (C) and in a hybrid micellar mobile phase containing 0.03 mol/l SDS in 10% methanol (D). Flow-rate, 1 ml/min; detection, UV, 254 nm. Analytes: phloroglucinol (1), hydroquinone (2), resorcinol (3), pyrocatechol (4) and phenol (5).

the retention time of the last eluted phenol was reduced from 17.4 to 9.9 min, while the resolution of the "critical pair" phloroglucinol and hydroquinone was the same as in aqueous SDS. In submicellar mobile phases with 0.003 mol/l SDS in 10% methanol, the separation is similar to that in the hybrid micellar phase shown in Fig. 9D, with time of separation increased to 20 min.

Another example illustrating the selectivity effects is the separation of a mixture of six barbiturates. In methanol–water mobile phases phenobarbital is not resolved from aprobarbital (Fig. 10A). The two solutes are well resolved in CTAB-containing micellar phases (Fig. 10B), but the time of separation is

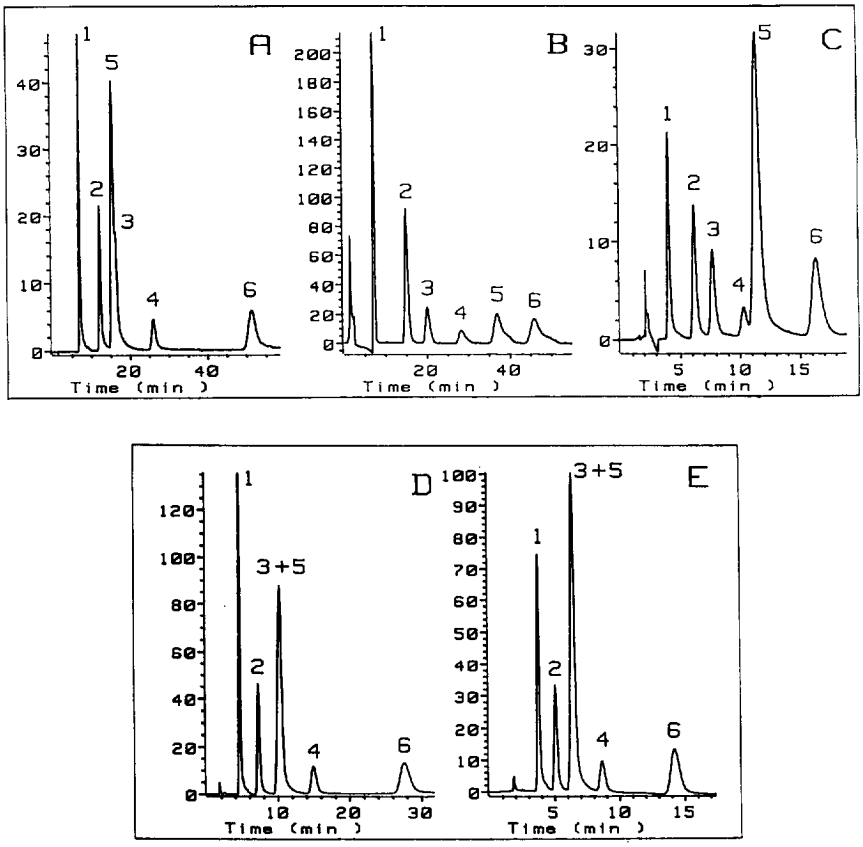


Fig. 10. Separation of a mixture of barbiturates on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. in 30% methanol in water (A), in a micellar mobile phase containing 0.02 mol/l CTAB in water (B) and in a submicellar mobile phase containing 0.03 mol/l CTAB in 30% methanol (C), in a hybrid micellar mobile phase containing 0.03 mol/l SDS in 30% methanol (D) and in a submicellar mobile phase containing 0.03 mol/l SDS in 40% methanol (E). Flow-rate and detection as in Fig. 9. Analytes: barbital (1), allobarbital (2), aprobarbital (3), butobarbital (4), phenobarbital (5) and pentobarbital (6).

long. The elution is accelerated in submicellar mobile phase containing 0.03 mol/l CTAB in 30% methanol, where the micelles are disaggregated (Fig. 10C). The relative retention of phenobarbital with respect to other barbiturates is decreased in this mobile phase and is in-between that in methanol–water and in aqueous CTAB mobile phases. The separation in micellar mobile phases containing 0.03 mol/l SDS in 30% methanol (Fig. 10D) is similar to aqueous methanol, with coelution of phenobarbital and aprobarbital, but the separation selectivity between the two solutes and allobarbital improves. Increasing the concentration of methanol to 40% causes transition from a hybrid micellar to a submicellar system, but the separation selectivity remains unchanged, while the time of separation decreases by 50% (Fig. 10E).

### *3.5. Methylene selectivity in micellar and submicellar phases*

The last point investigated was the methylene selectivity (i.e., the difference between the log $k$ of neighbouring homologous compounds), $\alpha$ in micellar and submicellar mobile phases. The dependence of $k$ on $n$ and on the concentration of methanol in the mobile phases that do not contain surfactants can be described by Eq. 7 [18]:

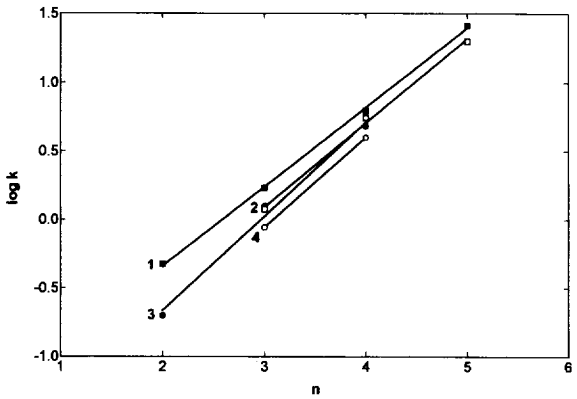


Fig. 11. Dependence of the capacity factor, $k$, on the number of carbon atoms, $n$, in the homologous $n$-alkanols (full markers, 1, 3) and iso-alkanols (empty markers, 2, 4) series on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in a submicellar mobile phase containing 0.003 mol/l SDS in water (squares, 1, 2) and in a micellar mobile phase containing 0.03 mol/l SDS in water (circles, 3, 4).

$$\log k = \log \beta + \log \alpha n$$
$$= a_0 + a_1 n - (m_0 + m_1 n)\varphi \quad (7)$$

The experimental plots of $\log k$ versus the number of carbon atoms in the alkyl chain, $n$, were linear both in micellar and in submicellar systems (Fig. 11), in agreement with Eq. 7 [18], but in contrast to the results of other workers [2], who claim a linear dependence of $k$ on $n$. The experimental data for homologous $n$- and iso-alkanols and homologous 3-$n$-alkyl-6-methyluracils are given in Table 8 and Table 9. The homologous selectivity $\alpha$ in $n$-alkanol series was slightly higher in the submicellar mobile phase containing 0.003 mol/l SDS in water with respect to the selectivity in the micellar mobile phase containing 0.03 mol/l SDS in water, but the opposite behaviour was found for the iso-isomers.

Similar behaviour was observed also in the homologous alkyluracil series, where $\log k$ increased

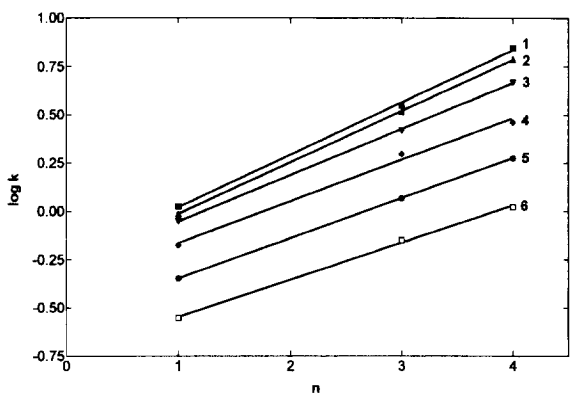


Fig. 12. Dependence of the capacity factor, $k$, on the number of carbon atoms, $n$, in the $n$-alkyl chains of homologous 3-methyl-6-$n$-alkyluracils series on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in micellar and submicellar range of concentrations of methanol in mobile phases containing 0.03 mol/l SDS and 0 (1), 10 (2), 20 (3), 30 (4), 40 (5) and 50% (6) methanol in water.

Table 8
Capacity factor $k$ of $n$- and iso-alkanols on s Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in a submicellar mobile phase containing 0.003 mol/l SDS in water and in a micellar mobile phase containing 0.03 mol/l SDS in water

| Compound | $k$ $c_s$=0.003 mol/l | | $k$ $c_s$ =0.03 mol/l | |
|---|---|---|---|---|
| Methanol | 0.19 | $n$-alkanols 2–5: | 0.20 | $n$-alkanols 2–4: |
| Ethanol | 0.47 | log $\beta$=−1.499 | 0.42 | log $\beta$=−1.454 |
| $n$-Propanol | 1.70 | log $\alpha$=0.579 | 1.26 | log $\alpha$ =0.530 |
| | | $r$=0.999 | | $r$=0.998 |
| Isopropanol | 1.19 | iso-alkanols 3–5: | 0.88 | iso-alkanols 3, 4 |
| $n$-Butanol | 6.24 | log $\beta$=−1.757 | 4.82 | log $\beta$=−2.022 |
| Isobutanol | 5.53 | log $\alpha$ =0.615 | 3.98 | log $\alpha$ =0.655 |
| n-Pentanol | 25.9 | $r$=0.999 | | |
| Isopentanol | 20.2 | | | |

log $\alpha$ and log $\beta$ are parameters of Eq. 7, $n$ is the number of carbon atoms and $r$ is the correlation coefficient.

Table 9
Capacity factor $k$ and parameters $\alpha$ and $\beta$ of Eq. 7 of 3-methyl-6-$n$-alkyluracils on s Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in a micellar (0–30% methanol) and submicellar (30–50% methanol) mobile phase containing 0.03 mol/l SDS in water and in a micellar mobile phase containing 0.03 mol/l SDS in water

| $\varphi_{MeOH}$ | $k$ for 3-methyl-6-$n$-alkyluracils | | | log $\beta$ | log $\alpha$ | $r$ |
|---|---|---|---|---|---|---|
| | 3.6-di-Me | 3-$n$-Pr-6-Me | 3-$n$-Bu-6-Me | | | |
| 0 | 1.06 | 3.54 | 7.02 | −0.252 | 0.272 | 0.999 |
| 0.1 | 0.97 | 3.27 | 6.14 | −0.281 | 0.267 | 0.999 |
| 0.2 | 0.89 | 2.62 | 4.68 | −0.292 | 0.239 | 0.999 |
| 0.3 | 0.67 | 1.98 | 2.90 | −0.378 | 0.215 | 0.997 |
| 0.4 | 0.45 | 1.17 | 1.89 | −0.555 | 0.208 | 0.999 |
| 0.5 | 0.28 | 0.71 | 1.05 | −0.741 | 0.193 | 0.999 |
| Eq. 1, $\varphi_{MeOH}$=0.1–0.3 | | | | Eq. 7, $\varphi_{MeOH}$=0.1–0.3 | | |
| $a_m$ | 0.081 | 0.628 | 0.966 | $a_0$=−0.220 | $m_0$=0.484 | |
| $m_{hyb}$ | −0.799 | −1.090 | −1.630 | $a_1$=0.292 | $m_1$=0.258 | |
| $r$ | 0.956 | 0.998 | 0.988 | | | |
| Eq. 2, $\varphi_{MeOH}$=0.3–0.5 | | | | Eq. 7, $\varphi_{MeOH}$=0.3–0.5 | | |
| $a$ | 0.402 | 0.962 | 1.132 | $a_0$=0.170 | $m_0$=1.816 | |
| $m$ | −1.900 | −2.225 | −2.204 | $a_1$=0.249 | $m_1$=0.110 | |
| $r$ | 0.999 | 0.999 | 0.998 | | | |

$a$ and $m$ are parameters of Eq. 2, $a_m$ and $m_{hyb}$ are parameters of Eq. 1, $a_0$, $a_1$, $m_0$ and $m_1$ are the parameters of Eq. 7, $n$ is the number of carbon atoms in the $n$-alkyl chain and $r$ is the correlation coefficient.

linearly with increasing $n$ and decreased as a linear function of the concentration of methanol in mobile phases containing 0.03 mol/l SDS in aqueous methanol, both in micellar (0–30%) and in submicellar (30–50%) range (Fig. 12). In contrast to reversed-phase chromatography in methanol–water mobile phases, where the homologous selectivity, $\alpha$, is approximately equal in different homologous series [18], we found slight differences between the values of $\alpha$ for $n$- and iso-alkanols (Table 8) and much more significant differences between the slopes of the dependencies for $n$-alkanols and for alkyluracils in micellar mobile phase comprised of 0.03 mol/l SDS in water (Table 8 and Table 9). The constants

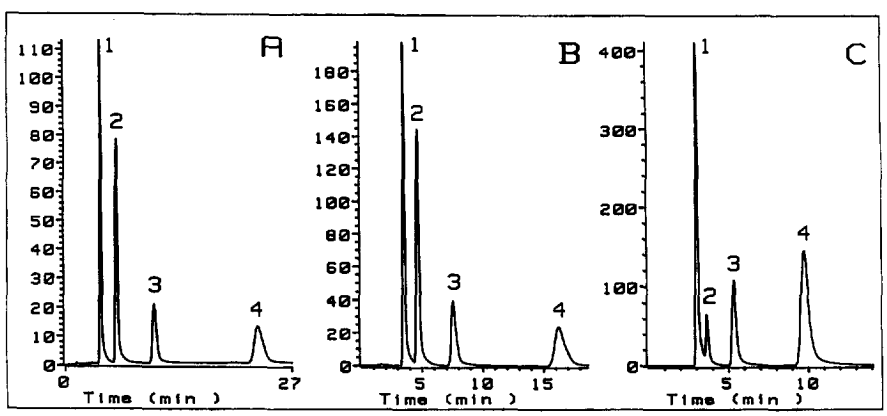


Fig. 13. Separation of a mixture of 3,6-dimethyl- (1), 3-methyl-6-ethyl- (2), 3-methyl-6-$n$-propyl- (3) and 3-methyl-6-$n$-butyl (4) uracils on a Silasorb SPH $C_8$, 7.5 $\mu$m, 300×3.6 mm I.D. column in 30% methanol in water (A), in a submicellar mobile phase containing 0.003 mol/l SDS in 30% methanol (B) and in a hybrid micellar mobile phase containing 0.03 mol/l SDS in 30% methanol (C). Flow-rate and detection as Fig. 9.

$a_0$, $a_1$, $m_0$ and $m_1$ in Eq. 7 in the alkyluracil series are different for micellar (30–50% methanol) and for submicellar (10–30% methanol) ranges of the SDS–methanol–water mobile phases (Table 9). Fig. 13 illustrates the effect of increasing concentration of SDS in 30% methanol on decreasing time of separation of lower uracils when the mobile phase is gradually changed from aqueous–organic (0% SDS, Fig. 13A) to submicellar (0.003 mol/l SDS, Fig. 13B) and hybrid micellar (0.03 mol/l SDS, Fig. 13C).

## 4. Conclusions

The CMC of CTAB, Septonex and SDS is affected by the addition of methanol to their aqueous solutions. There is a limiting concentration of methanol above which micelles do not occur. This concentration depends on the surfactant, and CTAB, Septonex and SDS do not form micelles in mobile phases with more than 20, 25 or 30% methanol, respectively. The CMC values of Septonex and SDS are practically unaffected by methanol in concentrations below 10% and increase as the concentration of methanol is further increased, whereas the CMC of CTAB increase as the concentration of methanol is raised from 0 to 10% and remain approximately constant in mobile phases containing 10–18% methanol.

The sorption isotherms of the surfactants on an octylsilica gel column in aqueous–methanolic solvents can be described using modified Langmuir equation in the concentration range below CMC. The amount of the adsorbed surfactant decreases as the concentration of methanol in aqueous–methanolic mobile phase is increased, but the rate of the decrease is significantly higher in submicellar than in micellar range of the mobile phases.

The dependencies of the capacity factors of the solutes studied on the concentration of methanol in the mobile phase can be suitably described by the same form of equation as that conventionally used for aqueous–organic mobile phases that do not contain surfactants, but the slopes of the dependencies for a given solute are different in micellar and submicellar ranges of the mobile phase composition. The ratio of the two slopes is controlled by the interaction of the analytes with the surfactant and with micelles and is approximately equal to, lower or higher than 1, depending on whether the solutes do or do not form associates with, or are repulsed from the micelles.

The retention in homologous series in aqueous–methanolic mobile phases containing a surfactant can be described by the same dependence of $\log k$ on the concentration of methanol and on the number of carbon atoms in the alkyl chains, but with different numerical values of the constants in the micellar and in the submicellar range.

The selectivity of separation may be adjusted by controlling simultaneously the concentrations of the organic solvent and of the surfactant in the mobile phase, which can be used for fine tuning of the separation as a complement to commonly used control of the concentrations of two organic solvents in ternary aqueous–organic mobile phases.

## Acknowledgments

This publication is based on work under Project No. 203/94/1397 sponsored by the Grant Agency of the Czech Republic.

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