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Characterisation of retention in micellar high-performance liquid chromatography and in micellar electrokinetic chromatography using lipophilicity and polarity indices

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Abstract

The retention of various barbiturates, phenylurea and triazine herbicides was measured in high-performance liquid chromatography (HPLC) on a C_8 column in aqueous micellar and non-micellar mobile phases containing anionic and cationic surfactants. The retention was characterised using the lipophilicity and polarity indices suggested earlier. The migration of the compounds tested was measured in the same mobile phases using micellar electrokinetic chromatography (MEKC) in fused-silica capillaries. The effects of the surfactants on the separation of uncharged compounds were less in the non-micellar than in the micellar region, but were still significant. The lipophilicity and polarity indices can be applied here, too, to characterise and predict the retention behaviour as a function of the concentration of the organic modifier and of the surfactant in the mobile phase or in the working electrolyte. Unlike the situation in micellar HPLC, the effects of the concentration of the surfactant on the lipophilicity selectivity in an homologous series are negligible in MEKC, where the polarity parameters may be used to characterise the contribution of the electrophoretic migration to the retention. The behaviour observed may possibly be explained by the lipophilic part of the surfactant molecules and of the organic solvent in aqueous–organic mobile phases having similar effects on the separation. The present approach offers the possibility of determining the critical micellar concentration from the migration times obtained using MEKC. © 1998 Elsevier Science B.V.

Keywords: Micellar liquid chromatography; Lipophilicity; Micellar electrokinetic chromatography; Polarity; Mobile phase composition; Buffer composition; Pesticides; Triazines; Phenylureas; Barbiturates; Alkanones

1. Introduction

Micellar liquid chromatography, first introduced in 1980 by Armstrong and Henry [1], differs from reversed-phase liquid chromatography (LC) in the mobile phase used, which contains a surfactant, such as SDS (sodium dodecyl sulphate) or CTAB (cetyltrimethylammonium bromide) at concentrations that are higher than the critical micellar concentration (CMC), so that a part of the surfactant is present in the form of molecular aggregates, i.e., micelles. The retention is controlled by the distribution of solute molecules between the aqueous mobile phase, the non-polar stationary phase and the micellar ''pseudo-stationary phase'' and the solutes are separated mainly on the basis of differences in their polarities [2–5], as in conventional reversed-phase chromatography with aqueous–organic solvents. The mobile phase in micellar liquid chromatography may not contain an organic solvent, but its addition is useful for decreasing the retention of strongly retained analytes. However, at high concentrations of organic

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solvent, the micelles disaggregate and the mobile phases contain only free surfactant molecules. Consequently, the effect of the concentration of the organic solvent on retention is different in the low-concentration (micellar) and in the high-concentration (non-micellar) regions [6–8].

In micellar electrokinetic chromatography (MEKC), developed by Terabe et al. [9,10], the potential applied across a fused-silica capillary is the driving force for migration, as in capillary zone electrophoresis. The difference between the two methods involves the addition (or not) of an anionic micellar additive (SDS) to the working electrolyte, which moves in the capillary from the anode (injector) to the cathode (detector) in the direction of the electroosmotic flow (EOF), but at the slowest velocity of all of the compounds migrating in the capillary. This allows the separation of nonionic compounds on the basis of their distribution between the aqueous electrolyte and the moving micellar ''pseudo-stationary phase'', so that less polar solutes migrate to the detector more slowly than more polar compounds, and all separated compounds are eluted in the ''retention window'' between the time of the EOF (the shortest time) and the migration time of the micelles (the longest time).

There are obvious similarities between the retention mechanisms controlling aqueous–organic reversed-phase chromatography, micellar liquid chromatography and MEKC. Hence, it is possible to correlate the retention in MEKC and the structure of the solutes, as in high-performance liquid chromatography (HPLC). A linear solvation energy relationship (LSER) characterises the solvent-related properties of solutes (i.e., k, log k, log P_{OW}) by the linear combination of the solvatochromic parameters: molar volume of solute, dipolarity/polarizability interactions with the solvent and the solute's basicity and acidity. As reported by Yang and Khaledi [11], LSER models for the retention of sixteen compounds using micellar liquid chromatography and of 25 compounds using MEKC resulted in a good agreement between the experimental and the calculated data. Good correlation was found between the n-octanol–water partition coefficients, log P_{OW}, used as a parameter of lipophilicity and retention indices for various compounds [12,13]. The retention index concept, widely used in gas chromatography (GC) [14] and in HPLC [15], has been applied in MEKC by Muijselaar et al. [16] for the characterisation of neutral aromatic compounds and of the retention properties of pseudo-stationary phases in MEKC [17]. The retention indices show better repeatability than the retention factors and are independent of the phase ratio, i.e., of the surfactant concentration, so that they are useful for identification of peaks in MEKC.

The objective of this work was to investigate the possibility of calibrating the retention scale in micellar liquid chromatography and in MEKC using the concept of the polarity and the lipophilicity indices, introduced earlier for this purpose in reversed-phase HPLC [18,19].

2. Theoretical

A simple method for the characterisation of retention in reversed-phase chromatography is based on the lipophilicity and polarity indices, n_{ce} and q_i, of solutes [19–21]. The lipophilicity index, n_{ce}, gives the hypothetical equivalent number of carbon atoms in the alkyl chain of an homologous calibration series and depends on the type of calibration series used. Ideally, it should not be significantly affected by the column packing material and by the type of organic solvent present in the mobile phase. The index, q_i, is a measure of the polarity of solute–solvent interactions and is expected to depend strongly on the organic solvent and (possibly) on polar groups in the stationary phase.

A linear dependence of the logarithm of the retention factor, k, on the volume fraction, φ, of the polar organic solvent in the binary aqueous–organic mobile phase is assumed [22,23]:

$$\log k = a - m\varphi \tag{1}$$

The indices scale is based on a suitable homologous calibration series, for which the parameters a and m depend on the number of carbon atoms, n:

$$\log k = a_0 + a_1 n - (m_0 + m_1 n)\varphi$$
$$= (a_0 + a_1 n)(1 - p\varphi) - q\varphi \tag{2}$$

as the parameters a and m of Eq. (1) in the homologous series are correlated:

$$m = q + pa \quad (3)$$

The general validity of Eq. (2) can be assumed, as each compound can be considered as a ''hypothetical'' member of the homologous calibration series, with its own indices n_{ce} and q_i, as the equivalents of the parameters n and q:

$$\log k = (a - a_1 n_{ce})(1 - p\varphi) - q_i\varphi \quad (4)$$

To calculate the indices n_{ce} and q_i for various non-homologous sample compounds, the constants a_0, a_1 and p of the homologous calibration series are introduced into Eqs. (5) and (6), together with the constants a and m of Eq. (1) for each sample compound:

$$n_{ce} = \frac{a - a_0}{a_1} \quad (5)$$

$$q_i = m - p(a_0 - a_1 n_{ce}) \quad (6)$$

As the effect of the concentration of methanol on retention is similar in aqueous–organic and in micellar reversed-phase chromatography [8], the lipophilicity and the polarity indices approach can be used for the calibration of retention, not only in conventional reversed-phase HPLC, but also in HPLC using micellar and non-micellar mobile phases containing surfactant additives and a polar organic solvent.

According to the commonly accepted mechanism of MEKC, the micellar ''pseudo-stationary phase'' in the working electrolyte theoretically has a similar effect on the retention as has the non-polar stationary phase in reversed-phase HPLC. The migration velocity of solutes in MEKC depends on the concentration of surfactant used in the micellar working electrolyte, c_{surf}, which controls the concentration of the micelles in the working electrolyte, c_{mic} ($c_{mic} = c_{surf} - \mathrm{CMC}$). The CMC is the critical micellar concentration, below which aggregated micelles do not form.

From the experimental data, a linear relationship was found between the logarithm of the retention factor, k, of the solute and the logarithm of the concentration of micelles in the working electrolyte

$$\log k = a + m \log c_{mic} \quad (7)$$

The separation mechanism for neutral compounds in MEKC is based on their partitioning between two moving phases, an electroosmotically driven aqueous phase and a micellar ''pseudo-stationary phase'':

$$K_D = \frac{[S]_{mic}}{[S]_{aq}} \quad (8)$$

Retention in MEKC can be adequately described by the retention factor, k [9,10]:

$$k = \frac{t_R - t_{EOF}}{t_{EOF}(1 - t_R/t_{MC})} \quad (9)$$

where t_R, t_{EOF} and t_{MC} are the migration times of the solute, of a neutral polar compound that is unretained in the micellar ''pseudo-stationary phase'' and migrating with the electroosmotic flow (EOF marker) and of a compound that is completely retained in the micellar ''pseudo-stationary phase'' (micelle marker), respectively. Rathore and Horváth [24] suggested a more complex formula, allowing one to calculate the overall retention factor of charged solutes in MEKC, where the electrophoretic migration contributes significantly to the migration process.

The retention factor in MEKC is a function of the concentration of micelles in the micellar working buffer

$$k = K_D \frac{V_S}{V_M} = K_D \frac{\mathrm{CONST}\, c_{mic}}{V_M} \quad (10)$$

where V_S is the volume of the micellar ''pseudo-stationary phase'' and V_M is the volume of the aqueous phase in the separation capillary. V_S is directly proportional to the concentration of micelles, $[S]_{mic} = c_{mic}$, with the proportionality constant, CONST. Using a logarithmic form of Eq. (9), we obtain Eq. (7), with the parameter $m = 1$.

$$\log k = a + \log c_{mic} = a + \log (c_{surf} - \mathrm{CMC}) \quad (11)$$

where $[S]_{aq} = c_{surf}$ is the total (analytical) concentration of surfactant in the working electrolyte. Using the same calibration approach as in reversed-phase chromatography, the retention of members of the homologous calibration series can be described as:

$$\log k = (a_0 + a_1 n)(1 + p \log c_{mic}) + q \log c_{mic} \quad (12)$$

and for non-homologous solutes, we obtain:

$$\log k = (a_0 + a_1 n_{ce})(1 + p \log c_{mic}) + q_i \log c_{mic} \quad (13)$$

where n_{ce} and q_i are the lipophilicity and the polarity indices calculated as in reversed-phase HPLC from the migration data of the calibration compounds and of the analytes, using Eqs. (2), (3), (5), (6).

Theoretically, values of $q = q_i = m_0 = 1$ and $p = m_1 = 0$ are expected from Eq. (11), if the distribution of the sample compound between the aqueous phase and the micellar ''pseudo-stationary phase'' is controlled only by the hydrophobic MEKC mechanism, and the values of the parameter n_{ce} characterise the hydrophobicity of the solute. If the experimental values of the parameter q_i differ from unity, they can be considered as a measure of non-ideal behaviour, i.e., of possible polar or ionic interactions or of the contribution of electrophoretic migration.

3. Experimental

3.1. Chemicals

Standards of phenylurea and triazine herbicides used as the tested compounds were obtained from Synthesia (Pardubice-Semtín, Czech Republic). Their structures are given in Table 1. Acetone, *n*-butan-2-one, *n*-pentan-2-one, *n*-hexan-2-one and *n*-octan-2-one (all of analytical grade) were obtained from Fluka (Buchs, Switzerland). Individual standards and their mixtures were dissolved at appropriate concentrations in the mobile phases or working electrolytes used.

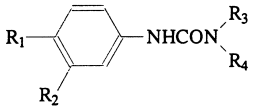
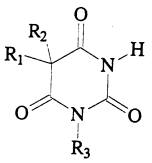
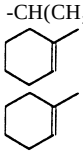
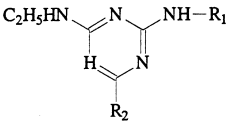
CTAB and SDS were obtained from Fluka, and sodium tetraborate, boric acid (both of analytical grade) and methanol (UV spectroscopy grade) were obtained from Lachema (Brno, Czech Republic). Water, used for preparation of the mobile phases and the working electrolytes, was double-distilled in glass with potassium permanganate and sodium bicarbonate. Mobile phases were prepared by mixing methanol, water and surfactant in the required proportions. The working electrolytes for MEKC were prepared by mixing the buffer components in appropriate ratios (an OP 208 pH meter, Radelkis, Budapest, Hungary, was used to monitor the pH) and then the required amount of surfactant was added to the buffer solution. All mobile phases and working electrolytes were filtered using a Millipore 0.45 μm filter and degassed by ultrasonication before use.

3.2. Apparatus

A HP 1090 M 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 for the chromatographic experiments. Two stainless steel columns (300×3.6 mm I.D.), packed in the laboratory with a spherical octylsilica sorbent, Silasorb SPH C_8, 7.5 μm (Lachema, Brno, Czech Republic), were used; one for mobile phases containing SDS and the other for mobile phases containing CTAB. The column temperature was kept at 35°C and the flow-rate of the mobile phase was 1 ml/min. The detector was operated at 230 nm, except for the ketones used as the standards for calibration of the retention (270 nm). The retention factors, k, of compounds were calculated from their retention volumes, V_R, and from the columns' dead volume, V_M, which was determined as the elution volume of unretained compound (methanol) detected at 200 nm, $k = (V_R - V_M)/V_M$.

A Crystal 310 capillary zone electrophoresis instrument (ATI Unicam, Cambridge, UK), equipped with a variable wavelength detector, was used for MEKC. Fused-silica capillaries, 75 cm long (60 cm effective length to the detector, 50 μm I.D.; J and W, Folsom, CA, USA) were subsequently washed with 0.1 mol/l NaOH (10 min), water (10 min) and working electrolyte (until a stabilised baseline was obtained) before use. The temperature of the capillary was set at 35°C. The separation was performed at a potential of +20 kV, which was applied across the capillary. The detection wavelengths were the same as those used in liquid chromatography. Methanol was used as the marker for electroosmotic flow time, t_{EOF}, and Sudan III azo dye {1-[4-(phenylazo)phenylazo]-2-naphthol, Lachema} was used as the marker for the migration time of micelles, t_{MC}. The retention factors of compounds were calculated from migration data using Eq. (9).

Table 1
Sample compounds and their structures

Compound	Basic structure	R_1	R_2	R_3	R_4
A. Phenylurea herbicides					
Phenylurea		-H	-H	-H	-H
Phenuron		-H	-H	$-CH_3$	$-CH_3$
Desphenuron		-H	-H	$-CH_3$	-H
Metoxuron		$-OCH_3$	-Cl	$-CH_3$	$-CH_3$
Deschlorometoxuron		$-OCH_3$	-H	$-CH_3$	$-CH_3$
Diuron		-Cl	-Cl	$-CH_3$	$-CH_3$
Chlorobromuron		-Br	-Cl	$-CH_3$	$-OCH_3$
Metobromuron		-Br	-H	$-CH_3$	$-OCH_3$
Isoproturon		$-CH(CH_3)_2$	-H	$-CH_3$	$-CH_3$
Fluometuron		-H	$-CF_3$	$-CH_3$	$-CH_3$
Monolinuron		-Cl	-H	$-CH_3$	$-OCH_3$
Linuron		-Cl	-Cl	$-CH_3$	$-OCH_3$
Neburon		-Cl	-Cl	-Cl	$-C_4H_9$
Cycluron	*N′*-cyclooctyl-*N*,*N*-dimethylurea				
B. Barbiturates					
Barbital		$-C_2H_5$	$-C_2H_5$	-H	–
Phenobarbital		$-C_2H_5$	-H	–	
Amobarbital		$-C_2H_4CH(CH_3)_2$	$-C_2H_5$	-H	–
Pentobarbital		$-CH(CH_3)C_3H_7$	$-C_2H_5$	-H	–
Cyclobarbital			$-C_2H_5$	-H	–
Hexobarbital			$-CH_3$	$-CH_3$	–
C. Triazine herbicides					
Simazine		$-C_2H_5$	-Cl	–	–
Atrazine		$-CH(CH_3)_2$	-Cl	–	–
Terbutryne		$-C(CH_3)_3$	$-SCH_3$	–	–

4. Results and discussion

4.1. Retention behaviour of homologous n-alkan-2-ones

The logarithms of retention factors of homologous calibration *n*-alkan-2-one standards decrease in a linear manner as the concentration of methanol in the mobile phase is increased, and they increase with increasing numbers of carbon atoms, *n*, in agreement with Eqs. (1) and (2) and with earlier results from aqueous–organic reversed-phase HPLC (Fig. 1). However, the slopes, *m*, of the log *k* versus φ plots are different in 10–30% methanol where the SDS micelles are present, and in 40–60% methanol where the SDS micelles cannot exist (Fig. 1B). Table 2 lists

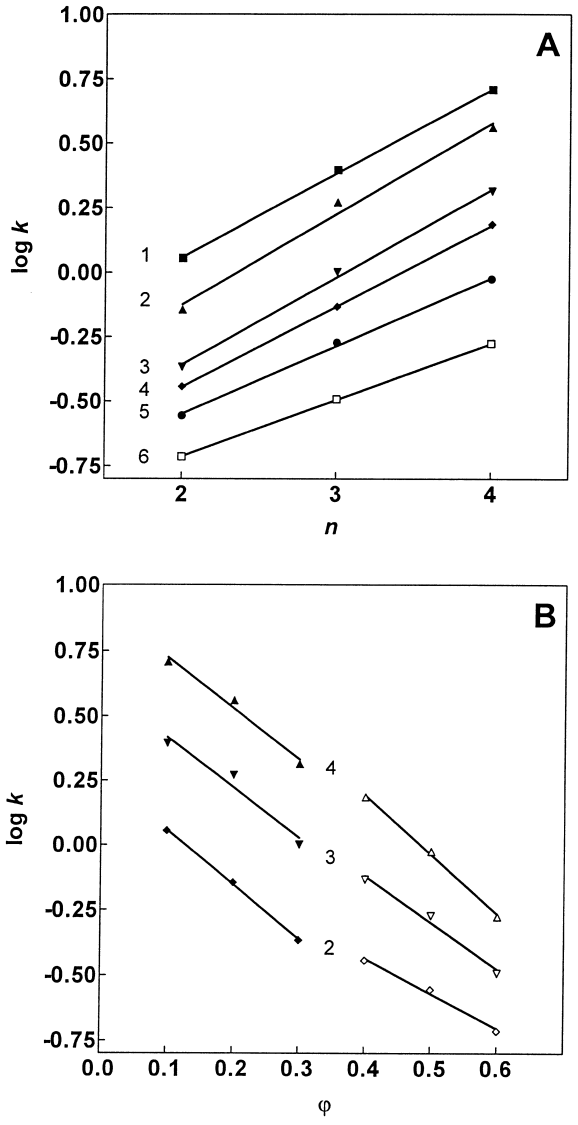


Fig. 1. Retention factors, k, of a homologous series of n-alkan-2-ones on a Silasorb SPH C_8 column in mobile phases with 0.1 mol/l SDS and different concentrations of methanol in water. (A) Dependence of log k on the number of carbon atoms, n, in n-alkyls in 1–10, 2–20, 3–30, 4–40, 5–50 and 6–60% (v/v) methanol. (B) Dependence of log k on the volume fraction, φ, of methanol in water (φ=% vol.$\times 10^{-2}$). Solid symbols, micellar range; open symbols, non-micellar range. Numbers of plots refer to the number of carbon atoms in the alkyl substituents.

the values of the parameters a_0, a_1, m_0, m_1, p and q of Eqs. (2) and (3) in the separation systems studied. The values of these parameters are similar in the non-micellar mobile phases containing SDS and CTAB and in aqueous–methanolic mobile phases without surfactants. In the SDS-containing mobile phases, the slopes, a_1, of the a versus n plots are higher in the micellar then in the non-micellar region (Fig. 2A). The slopes, m_1, of the m versus n plots are positive in the non-micellar, but negative in the micellar region of methanol concentrations (Fig. 2B). Consequently, the slope, p, of the dependencies of m on a (Eq. (3)) is positive in the non-micellar, but negative in the micellar region (Fig. 2C). In the micellar region, the m versus n and the m versus a plots are slightly curved, but in the non-micellar region, good linearity was observed, in agreement with Eqs. (2) and (3). The parameter q is significantly higher in the micellar than in the non-micellar phases containing SDS.

The experimental behaviour can be explained as follows: In the non-micellar range, the organic solvent does not affect the free molecules of the surfactant and its effect on the retention is very similar to that in reversed-phase chromatography with aqueous–organic mobile phases that do not contain surfactants, where the values of m_1 and p are always positive. However, in the micellar region, methanol contributes to disaggregation of the micelles, so that the concentration of the micelles decreases with increasing concentration of methanol. The retention of uncharged compounds in micellar HPLC generally increases as the concentration of the micelles decreases, so that this effect modifies the effect of the concentration of methanol on the retention, and the slopes, m, of Eq. (1) are consequently lower in the micellar than in the non-micellar region. As the affinity of the solutes to the micelles increases with increasing length of the alkyl chain in a homologous series, the effect of the disaggregation of the micelles is larger with higher than with lower oligomers, which explains the negative value of the parameter m_1 in the micellar region, and the decreasing slopes of the plots in Fig. 2B–C. In the absence of methanol, the micelles decrease the retention (a = log k) of higher oligomers more strongly than the free molecules of SDS, whereas the opposite holds true for the lower oligomers. Fig. 2A shows that the effect of the micelles and of the free molecules of SDS on the retention are approximately equal with n-pentan-2-one (n=3).

Table 2
Parameters a_0, a_1 and m_0, m_1 of Eq. (2) and parameters p and q of Eq. (3) of the homologous n-alkan-2-one calibration series in the separation systems tested: Dependence on the concentration of methanol in the mobile phase in HPLC; dependence on the concentration of the SDS micelles in the MEKC working electrolytes

System	Eq. (2)			Eq. (2)			Eq. (3)		
	a_0	a_1	R	m_0	m_1	R	p	q	R
Non-micellar, C_8 0.02 mol/l CTAB	−0.876	0.530	0.990	0.657	0.432	0.999	0.815	1.372	0.999
Non-micellar, C_8 0.03 mol/l CTAB	−0.611	0.400	0.998	1.073	0.186	0.988	0.668	1.193	0.997
Micellar, C_8 0.1 mol/l SDS	−0.193	0.260	0.990	2.228	−0.092	0.971	−0.343	2.211	0.948
Non-micellar, C_8 0.1 mol/l SDS	−0.586	0.389	0.987	0.953	0.273	0.927	0.730	1.342	0.975
MEKC borate buffer	−0.047	0.377	0.994	1.163	−0.027	−	−0.063	1.149	−
Reversed-phase HPLC, C_{18} methanol–water	−0.561	0.605	0.999	0.922	0.499	0.934	0.828	1.372	0.998

R, correlation coefficients.

Similar behaviour as in the systems with SDS was also found in non-micellar phases containing CTAB. The values of the parameters a_1, m_1, p and q in non-micellar phases containing 0.02 mol/l CTAB are very close to the values determined in aqueous–methanolic mobile phases without surfactant; the values of a_1, m_1 and p decrease as the concentration of CTAB is increased. The values of these parameters in non-micellar mobile phases with 0.1 mol/l SDS are close to the values in non-micellar phases with 0.03 mol/l CTAB. As CTAB micelles are not formed in mobile phases containing more than 17% methanol, and the retention of the compounds tested was too strong in mobile phases with lower concentrations of methanol, it was not possible to determine the dependencies of the retention factors on the concentration of methanol in the micellar region.

4.2. Calibration of the retention scale in micellar and non-micellar HPLC systems

The experimental parameters, a and m, of Eq. (1) for phenylurea herbicides in micellar and non-micellar phases containing CTAB and SDS are given in Tables 3 and 4. Good linearity of the log k values versus the concentration of methanol, φ, was found, as in aqueous–methanolic mobile phases without CTAB (Table 5). The slopes, m, of these plots are higher in the micellar than in the non-micellar range in mobile phases containing SDS, as was the case in the homologous n-alkan-2-one series, and they decrease as the concentration of CTAB increases in non-micellar mobile phases. This means that the effect of methanol on the retention behaviour decreases as the concentration of the free molecules of CTAB in the mobile phase increases.

The values of the lipophilicity and the polarity indices, determined using Eqs. (5) and (6), with the calibration standard n-alkan-2-one series, are similar in mobile phases with different concentrations of CTAB (Tables 3–5), but are, in most cases, higher than in the mobile phases with SDS. Higher values of the lipophilicity indices, n_{ce}, in non-micellar mobile phases with CTAB, in comparison to the mobile phases containing SDS, can possibly be explained by modification of the chemically bonded octylsilica phase by the CTAB adsorbed on the

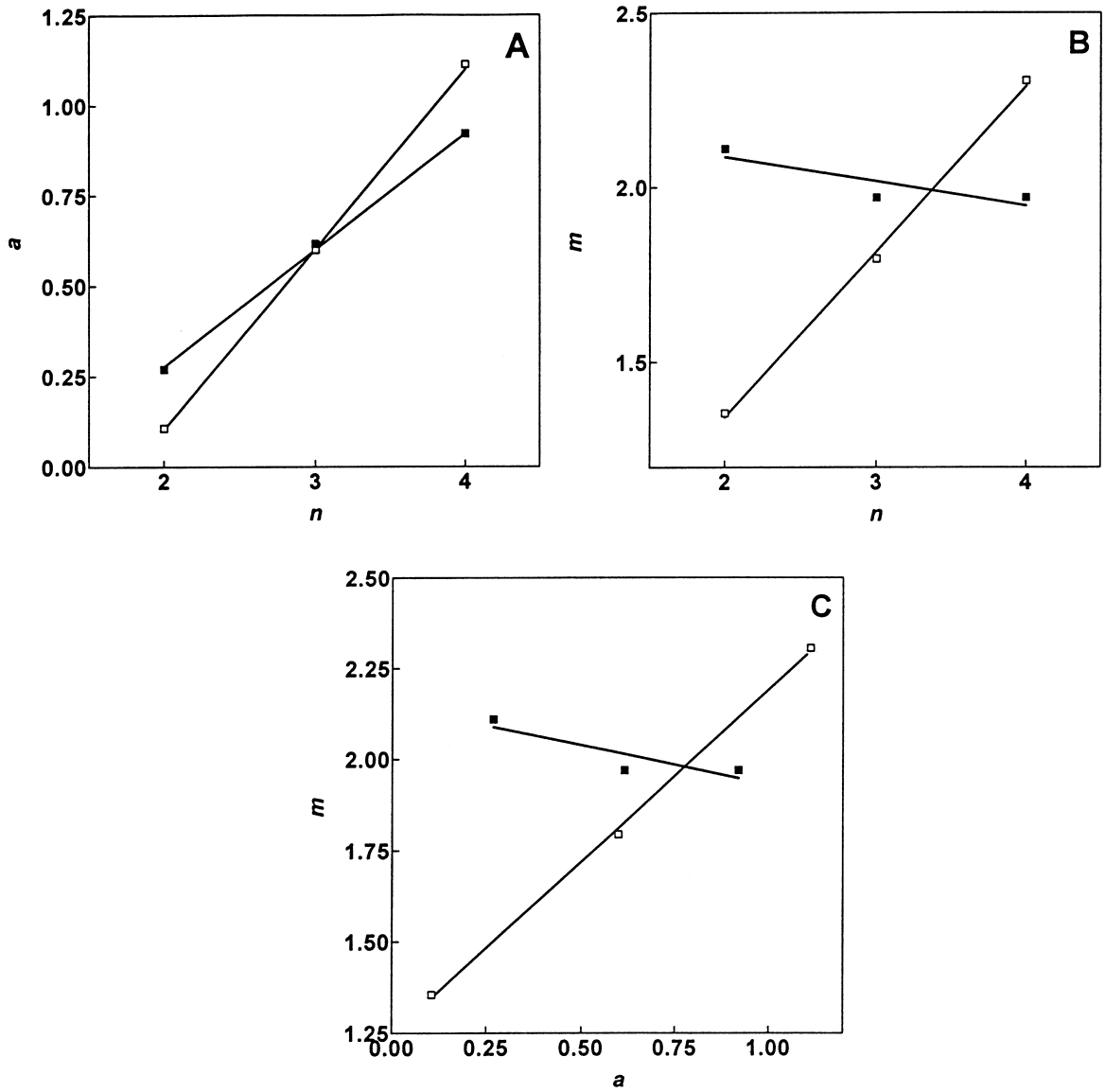


Fig. 2. Dependence of the intercept a (A) and of the slope m (B) of Eq. (1) on the number of carbon atoms in the alkyls of the homologous series of *n*-alkan-2-ones in micellar and non-micellar mobile phases and the correlation between the slope m and the intercept a of Eq. (3) for an homologous series of *n*-alkan-2-ones in micellar and non-micellar mobile phases (C). Conditions as in Fig. 1. Solid symbols, micellar range; open symbols, non-micellar range.

stationary phase, changing its properties by neutralising possible effects of the residual silanol groups and by increasing the amount of the non-polar moiety in the stationary phase. With the exception of monolinuron, the indices, q_i, are similar in the mobile phases containing SDS and CTAB at non-micellar concentrations. In the micellar range of the mobile phases containing SDS, the values of both lipophilicity and polarity indices are higher than in the non-micellar range (Table 4), except for metobromuron, linuron and neburon, the n_{ce} indices of which are almost equal in the two ranges. This

Table 3
Parameters a and m of the retention equation, Eq. (1) (φ = % vol.$\times 10^{-2}$ of methanol), and the lipophilicity and polarity indices, n_{ce} and q_i, of phenylurea herbicides on a Silasorb SPH C_8 column with non-micellar aqueous–methanolic mobile phases containing 0.02 (A) and 0.03 (B) mol/l CTAB

Mobile phase	Compound	a	m	R	n_{ce}	q_i
A	Phenuron	1.165	2.644	[a]	3.85	1.69
A	Diuron	2.317	3.059	[a]	6.02	1.17
A	Chlorobromuron	2.682	3.581	0.992	6.71	1.40
A	Metobromuron	2.302	3.497	0.996	5.99	1.62
A	Isoproturon	2.268	3.386	[a]	5.93	1.54
A	Fluometuron	2.305	3.648	[a]	5.71	1.54
A	Monolinuron	2.114	3.360	[a]	5.64	1.64
A	Linuron	2.456	3.153	[a]	6.28	1.15
B	Phenuron	0.928	2.011	0.997	3.84	1.58
B	Diuron	1.874	2.203	0.992	6.21	1.32
B	Chlorobromuron	2.045	2.298	[a]	6.64	1.34
B	Metobromuron	1.858	2.615	0.996	6.17	1.75
B	Isoproturon	1.754	2.342	0.990	5.91	1.52
B	Fluometuron	1.737	2.426	0.991	5.87	1.62
B	Monolinuron	1.647	2.343	0.986	5.64	1.57
B	Linuron	1.848	2.004	0.992	6.14	1.14

Methanol concentration ranged from 40–60% (v/v) (A) and 20–50% (v/v) (B).
The *n*-alkan-2-one homologous series was used as the calibration standards.
R, correlation coefficient.
[a] Parameters a and m were calculated using only two points.

Table 4
Parameters a and m of the retention equation, Eq. (1) (φ = % vol.$\times 10^{-2}$ of methanol), and the lipophilicity and polarity indices, n_{ce} and q_i, of phenylurea herbicides on a Silasorb SPH C_8 column with micellar (A) and non-micellar (B) aqueous–methanolic mobile phases containing 0.1 mol/l SDS

Mobile phase	Compound	a	m	R	n_{ce}	q_i
A	Phenuron	0.819	2.825	0.999	3.89	3.11
A	Diuron	1.219	1.975	0.999	5.43	2.39
A	Chlorobromuron	1.225	1.545	0.999	5.45	1.96
A	Metobromuron	1.128	1.705	0.999	5.08	2.09
A	Isoproturon	1.355	2.360	0.999	5.95	2.82
A	Fluometuron	1.125	2.070	0.999	5.07	2.46
A	Monolinuron	0.979	1.810	0.971	4.51	2.15
A	Linuron	1.269	1.830	0.999	5.62	2.27
A	Neburon	1.425	1.480	0.999	6.22	1.97
B	Phenuron	0.630	2.105	0.993	3.13	1.65
B	Diuron	1.415	2.245	0.993	5.14	1.21
B	Chlorobromuron	1.468	2.075	0.994	5.28	1.00
B	Metobromuron	1.450	2.535	0.992	5.23	1.48
B	Isoproturon	1.443	2.380	0.999	5.21	1.33
B	Fluometuron	1.275	2.290	0.998	4.78	1.36
B	Monolinuron	1.038	1.915	0.967	4.17	1.16
B	Linuron	1.500	2.230	0.993	5.56	1.14
B	Neburon	1.862	2.410	0.989	6.29	1.05

The concentration of methanol ranged from 10–30% (v/v) (A) and 40–60% (v/v) (B).
The *n*-alkan-2-one homologous series was used as the calibration standards.
R, correlation coefficient.

Table 5
Parameters a and m of the retention equation, Eq. (1) (φ=% vol.$\times 10^{-2}$ of methanol), and the lipophilicity and polarity indices, n_{ce} and q_i, of triazine and phenylurea herbicides on a Silasorb C_{18} column with aqueous–methanolic mobile phases

Compound	a	m	R	n_{ce}	q_i
Phenuron	1.451	3.055	0.999	3.33	1.85
Desphenuron	1.202	2.576	0.997	2.93	1.58
Deschlorometoxuron	1.601	3.577	0.998	3.83	2.25
Metoxuron	2.382	4.576	0.999	4.87	2.61
Phenylurea	1.109	2.789	0.999	2.76	1.88
Linuron	3.441	4.758	[a]	6.62	1.91
Simazine	2.436	3.987	0.997	4.96	1.97
Atrazine	2.876	4.228	0.999	5.68	1.85

Methanol concentration 25–50% (v/v).
The *n*-alkan-2-one homologous series was used as the calibration standards.
R, correlation coefficient.
[a] The parameters a and m were calculated from two points only.

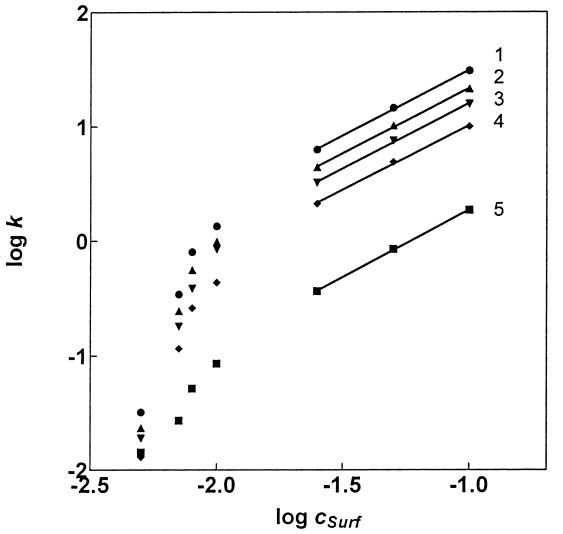


Fig. 3. Dependence of the retention factors, k, of phenylurea herbicides in MEKC on the analytical concentration of SDS, c_{surf}, in mol/l, in the borate working electrolyte (0.025 mol/l, pH 8.5). For other conditions, see Section 3. Compounds: 1, linuron; 2, diuron; 3, chlorotoluron; 4, fluometuron and 5, phenuron.

means that the lipophilicity and the polarity indices cannot be transferred from non-micellar into micellar systems.

4.3. Calibration of the retention scale in MEKC

The retention factors of the compounds tested increase with increasing concentration of SDS in the micellar range in MEKC, whereas their values are significantly lower in the non-micellar than in the micellar region (Fig. 3). In the micellar range, the slopes of the log k of the herbicides tested versus log c_{mic} plots are close to unity, as expected from Eq. (11).

The values of the parameters a_0, m_1 and p of *n*-alkan-2-ones in MEKC are very close to zero and the parameters m_0 and q are close to unity, as expected (Table 2). Consequently, the parameter, n_{ce}, of the sample compounds tested $n_{ce} \cong a/a_1$. The values of the parameter, q_i, determined using Eq. (6) is close to unity for phenylurea and triazine herbicides, but are significantly lower for barbiturates, which are weak acids and are partially ionised in the working electrolyte buffered at pH 8.5, which indicates that their migration to the detector is accelerated by the contribution of the electrophoretic migration (Table 6). This effect is also apparent from the low values of the parameter m of Eq. (1), whereas m is close to unity, as expected, for the herbicide compounds. The values of the lipophilicity indices of the herbicides studied, n_{ce}, in MEKC are close to the values of these indices determined in reversed-phase chromatography with aqueous–methanolic mobile phases, except for phenylurea, desphenuron, simazine and atrazine, which are 0.3–0.5 units higher in the MEKC systems.

In Table 7, experimental retention factors of the sample compounds, measured using MEKC with working electrolytes containing various micellar concentrations of SDS, are compared with the values calculated from Eq. (13) using the lipophilicity and polarity indices from Table 6. Very good agreement with the experimental data was observed for the retention data predicted by calculation from the indices, which means that this method of calibration of retention is adequate for MEKC systems with micellar SDS systems. However, this method cannot be used for MEKC separations using working electrolytes containing SDS at concentrations lower than the CMC.

Figs. 4–6 illustrate the separation of several

Table 6
Parameters a and m of the retention equation, Eq. (11) ($c_{mic} = c_{surf} - CMC$), and the lipophilicity and polarity indices, n_{ce} and q_i, of triazine and phenylurea herbicides and barbiturates in micellar electrokinetic chromatography with electrolytes containing various concentrations of SDS (0.025–0.1 mol/l) in aqueous borate buffer (0.025 mol/l, pH=8.5).

Compound	c_{surf}				
	a	m	R	n_{ce}	q_i
Phenuron	1.260	0.962	0.999	3.37	1.04
Desphenuron	1.264	0.937	0.999	3.48	1.02
Deschlorometoxuron	1.386	0.927	0.999	3.80	1.01
Metoxuron	1.815	0.917	0.999	4.94	1.03
Phenylurea	1.215	0.967	0.999	3.35	1.04
Linuron	2.464	0.943	1.000	6.66	1.10
Diuron	2.296	0.936	0.999	6.22	1.08
Chlorobromuron	2.608	0.943	0.999	7.04	1.11
Metobromuron	2.057	0.933	0.999	5.58	1.06
Isoproturon	2.258	0.947	0.999	6.12	1.09
Fluometuron	1.961	0.925	0.999	5.33	1.05
Monolinuron	1.914	0.940	0.999	5.20	1.06
Neburon	3.154	0.983	0.999	8.49	1.18
Simazine	1.938	0.924	0.999	5.27	1.05
Atrazine	2.263	0.947	0.999	6.13	1.09
Barbital	0.096	0.078	0.971	0.38	0.08
Phenobarbital	0.327	0.174	0.949	0.99	0.19
Cyclobarbital	0.540	0.332	0.988	1.56	0.36
Amobarbital	1.020	0.531	0.999	2.83	0.60
Pentobarbital	1.382	0.700	0.996	3.79	0.79
Hexobarbital	1.415	0.766	0.996	3.88	0.86

The *n*-alkan-2-one homologous series was used as the calibration standards.
R, correlation coefficient.

phenylurea herbicides in micellar HPLC and in MEKC. Similar elution orders were observed for these three separation systems, but the selectivities of separation were different in each system. The best separation was obtained in the MEKC system with micellar concentrations of SDS, where eleven compounds could be resolved in less than 30 min (Fig. 4), which is similar to the separation that can be obtained in gradient-elution reversed-phase HPLC. However, even in the electrolytes with SDS concentrations that were approximately equal to the CMC (0.01 mol/l), it was possible to separate nine herbicides in less than 10 min (Fig. 5). This compares favourably with the separation by HPLC with hybrid non-micellar phases containing SDS and 50% methanol, where the micelles disaggregate (Fig. 6).

Table 7
Comparison between the experimental (k_{exp}) retention factors and the calculated (k_{calc}) retention factors from the lipophilicity and polarity indices, n_{ce} and q_i, for triazine and phenylurea herbicides and barbiturates in micellar electrokinetic chromatography with electrolytes containing various concentrations of SDS (0.025–0.1 mol/l) c_{surf} in aqueous borate buffer (0.025 mol/l, pH=8.5)

Compound		c_{surf} (mol/l)		
		0.025	0.05	0.1
Phenuron	k_{exp}	0.366	0.847	1.855
	k_{calc}	0.363	0.864	1.835
Desphenuron	k_{exp}	0.408	0.930	1.980
	k_{calc}	0.405	0.943	1.965
Deschlorometoxuron	k_{exp}	0.563	1.276	2.685
	k_{calc}	0.560	1.291	2.667
Metoxuron	k_{exp}	1.561	3.589	7.310
	k_{calc}	1.564	3.574	7.327
Phenylurea	k_{exp}	0.323	0.752	1.649
	k_{calc}	0.320	0.766	1.633
Linuron	k_{exp}	6.281	14.55	30.76
	k_{calc}	6.260	14.65	30.65
Diuron	k_{exp}	4.404	10.15	21.30
	k_{calc}	4.394	10.21	21.24
Chlorobromuron	k_{exp}	8.763	20.33	42.92
	k_{calc}	8.739	20.45	42.79
Metobromuron	k_{exp}	2.570	5.930	12.36
	k_{calc}	2.566	5.947	12.34
Isoproturon	k_{exp}	3.865	8.889	19.07
	k_{calc}	3.839	9.015	18.93
Fluometuron	k_{exp}	2.177	4.903	10.04
	k_{calc}	2.122	4.881	10.07
Monolinuron	k_{exp}	1.789	4.109	8.762
	k_{calc}	1.787	4.167	8.696
Neburon	k_{exp}	25.68	65.82	134.0
	k_{calc}	26.14	63.38	136.8
Simazine	k_{exp}	2.020	4.649	9.573
	k_{calc}	2.021	4.646	9.578
Atrazine	k_{exp}	3.867	9.219	19.04
	k_{calc}	3.886	9.122	19.15
Barbital	k_{exp}	0.914	0.956	1.044
	k_{calc}	0.906	0.973	1.034
Phenobarbital	k_{exp}	1.060	1.140	1.424
	k_{calc}	1.048	1.255	1.404
Cyclobarbital	k_{exp}	0.956	1.187	1.650
	k_{calc}	0.934	1.248	1.606
Amobarbital	k_{exp}	1.219	1.914	2.984
	k_{calc}	1.209	1.950	2.955
Pentobarbital	k_{exp}	1.429	2.485	4.663
	k_{calc}	1.394	2.620	4.523
Hexobarbital	k_{exp}	1.186	2.149	4.329
	k_{calc}	1.151	2.294	4.179

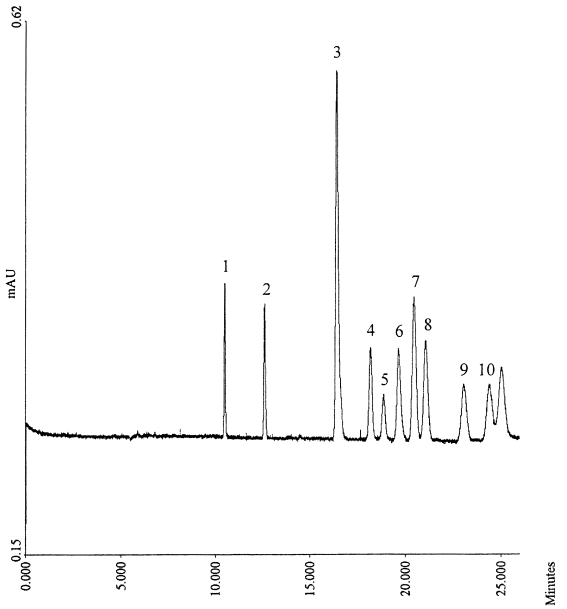


Fig. 4. MEKC separation of a mixture of phenylurea herbicides. Capillary, uncoated fused-silica, 75 (60) cm×50 μm I.D. Working electrolyte, 0.1 mol/l SDS in 0.025 mol/l borate (pH 8.5). Voltage, +20 kV. Compounds: 1, phenylurea; 2, deschlorometoxuron; 3, monuron; 4, monolinuron; 5, fluometuron; 6, *N*-phenyl-*N′*-butylurea; 7, chlorotoluron; 8, isoproturon; 9, chlorobromuron and 10, neburon.

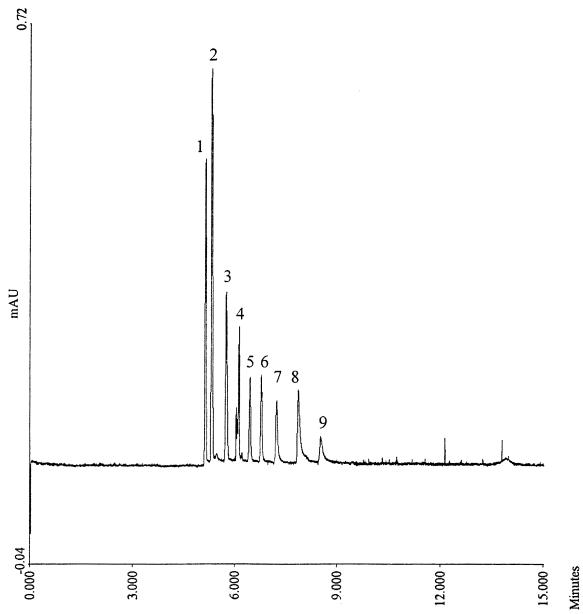


Fig. 5. CZE separation of a mixture of phenylurea herbicides using a working electrolyte with 0.01 mol/l SDS in 0.025 mol/l borate (pH 8.5). Capillary, uncoated fused-silica, 75 (60) cm×50 μm I.D. voltage, +20 kV. Compounds: 1, phenylurea; 2, deschlorometoxuron; 3, monuron; 4, monolinuron; 5, *N*-phenyl-*N′*-butylurea; 6, chlorotoluron; 7, diuron; 8, linuron and 9, chlorobromuron.

4.4. Possibilities for the determination of the CMC from the MEKC migration data

Eq. (11) can be rearranged into the form:

$$k' = 10^{a}(c_{surf} - \mathrm{CMC}) = -10^{a}\mathrm{CMC} + 10^{a}c_{surf} = a' + b'c_{surf} \quad (14)$$

Eq. (14) offers the possibility of determining the CMC from the dependence of the capacity factors in MEKC on the concentration of surfactant in the mobile phase, as the ratio of the *y*-axis intercept, a', and the slope, b', of this dependence is:

$$\mathrm{CMC} = -\frac{a'}{b'} \quad (15)$$

The validity of Eqs. (14) and (15) is illustrated by the data in Table 8. As expected, the plots of k versus c_{surf} are linear within the micellar concentration range. The correlation coefficients for Eq. (14) are very close to unity. The CMC calculated

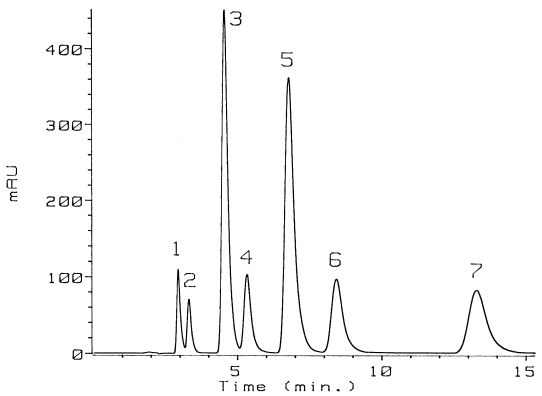


Fig. 6. Liquid chromatographic separation of a mixture of phenylurea herbicides. Column, Silasorb SPH C_8, 7.5 μm, 300×3.6 mm I.D. Mobile phase, non-micellar, 0.1 mol/l SDS in methanol–water (50:50, v/v), 1 ml/min. Compounds: 1, deschlorometoxuron; 2, desphenuron; 3, monuron; 4, fluometuron; 5, diuron; 6, chlorobromuron and 7, neburon.

Table 8
MEKC retention factors, k, and parameters a' and b' of Eq. (13), which was used for the calculation of the CMC, and calculated values of the CMC for SDS with less polar *n*-alkan-2-ones and herbicides

Compound	k at c_{surf} (mol/l)			a'	b'	R	CMC (mol/l)
	0.1	0.05	0.025				
n-Pentan-2-one	0.880	0.377	0.149	−0.103	9.791	0.9997	0.0105
n-Hexan-2-one	0.177	0.965	0.392	−0.214	23.863	0.9999	0.0089
n-Octan-2-one	15.826	6.975	2.786	−1.654	174.51	0.9988	0.0095
Terbutryne	119.88	56.789	22.008	−9.538	1298.8	0.9997	0.0073
Neburon	134.05	65.820	25.684	−8.429	1433.4	0.9991	0.0071
Cycluron	15.375	7.082	3.014	−1.133	164.96	0.9999	0.0069
CMC	Arithmetic mean						0.0084±0.0014
	From refractometric measurements						0.0079

R, correlation coefficient.

from the ratios of the experimental parameters a' and b' for different, less polar, ketones and herbicides, agree with each other and with the CMC values determined from the refractometric measurements. For more polar compounds, lower correlation coefficients and higher CMC values were obtained. The CMC values were close to each other for compounds that yield correlation coefficients of 0.999 or higher (only these data are given in Table 8).

5. Conclusions

The effect of methanol on the retention in micellar chromatography is similar to that in aqueous–organic reversed-phase chromatography without surfactants, but the slopes of the logarithms of the retention factors versus the concentration of methanol are different in mobile phases containing surfactant and an organic solvent, at concentrations where micelles exist and in the hybrid non-micellar mobile phases where the micelles disaggregate. Calibration of the retention using the lipophilicity and polarity indices with the homologous *n*-alkan-2-one series can be applied in micellar HPLC, but better agreement between the indices determined in aqueous–organic mobile phases is obtained for mobile phases containing surfactants at concentrations lower than the CMC than in real micellar mobile phases.

This calibration approach can also be used in MEKC, where deviations of the value of the polarity index, q_i, from unity indicate non-ideal behaviour effects, such as an electrophoretic contribution to the mobility of partially ionised compounds. From the dependence of the retention factors of less polar compounds on the concentration of surfactant in MEKC, the CMC can be determined.

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References

[1] D.W. Armstrong, S.J. Henry, J. Liq. Chromatogr. 3 (1980) 657.
[2] D.W. Armstrong, F. Nome, Anal. Chem. 53 (1981) 1662.
[3] M. Arunyanart, L.J. Cline-Love, Anal. Chem. 56 (1984) 1557.
[4] M.F. Borgerding, F.H. Quina, W.L. Hinze, J. Bowermaster, H.M. McNair, Anal. Chem. 60 (1988) 2520.
[5] P. Jandera, J. Fischer, J. Chromatogr. A 728 (1996) 279.
[6] M.G. Khaledi, E. Peuler, J. Ngeh-Ngwainbi, Anal. Chem. 59 (1987) 2738.
[7] A.S. Kord, M.G. Khaledi, J. Chromatogr. 631 (1993) 125.
[8] J. Fischer, P. Jandera, J. Chromatogr. B 681 (1996) 3.
[9] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[10] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[11] S. Yang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 301.
[12] Y. Ishihama, Y. Oda, N. Asakawa, Anal. Chem. 68 (1996) 1028.

[13] S. Yang, J.G. Bumgarner, L.F.R. Kruk, M.G. Khaledi, J. Chromatogr. A 721 (1996) 323.
[14] V. Pacáková, L. Feltl, Chromatographic Retention Indices, Ellis Horwood, New York, 1992.
[15] R.M. Smith, Adv. Chromatogr. 26 (1987) 277.
[16] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, Anal. Chem. 66 (1994) 635.
[17] P.G. Muijselaar, J. Chromatogr. A 780 (1997) 117.
[18] P. Jandera, Chromatographia 19 (1984) 101.
[19] P. Jandera, in R.M. Smith (Editor), Retention and Selectivity in Liquid Chromatography, Elsevier, Amsterdam, 1995, Ch. 8, p. 269.
[20] P. Jandera, J. Chromatogr. 352 (1986) 91.
[21] P. Jandera, J. Rozkošná, J. Chromatogr. 556 (1991) 145.
[22] P. Jandera, J. Churáček, J. Chromatogr. 91 (1974) 207.
[23] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3.
[24] A.S. Rathore, Cs. Horváth, J. Chromatogr. A 743 (1996) 231.