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Characterisation of retention in micellar high-performance liquid chromatography, in micellar electrokinetic chromatography and in micellar electrokinetic chromatography with reduced flow

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Abstract

The retention (migration) behaviour of various barbiturates, phenylurea and triazine herbicides in micellar electrokinetic chromatography (MEKC) with uncoated fused-silica capillaries was compared with the behaviour in micellar electrokinetic chromatography with reduced electroosmotic flow (RF-MEKC) using capillaries modified with linear polyacrylamide. The error in the values of the retention factors caused by the neglection of the contribution of the electroosmotic flow in RF-MEKC was investigated and a method for correcting this error was suggested. The retention was characterised using the lipophilic and polar indices to characterise and to predict the retention as a function of the concentration of the surfactant (sodium dodecylsulphate) in the running buffer in MEKC and in RF-MEKC. Homologous series of *n*-alkylbenzenes and of *n*-alkan-2-ones were compared as the standard sets for the calibration of the retention (migration) index scale. The values of the lipophilic indices of a given solute measured in reversed-phase HPLC, MEKC and RF-MEKC are close to each other. Under ideal MEKC conditions, the values of the polarity indices are close to one for various sample solutes. However, for partially ionised compounds such as weakly acidic barbiturates, where the contribution of the electrophoretic migration is significant, the values of the polarity indices are significantly lower than one. Optimum conditions for separations of mixtures of triazine and phenylurea herbicides and of barbiturates using various techniques tested were compared.

Keywords: Micellar electrokinetic chromatography; Micellar liquid chromatography; Electroosmotic flow; Retention indices; Barbiturates; Pesticides; Triazines; Phenylureas

1. Introduction

Micellar liquid chromatography (MLC) differs from reversed-phase liquid chromatography (RP-HPLC) by the mobile phase, which contains a surfactant such as SDS (sodium dodecylsulphate) or CTAB (cetyltrimethylammonium bromide) at a concentration higher than the critical micellar concentration, CMC, so that a part of the surfactant is present in the form of molecular aggregates — micelles. The retention is controlled by the distribution of the molecules of analytes between the aqueous mobile phase, the non-polar stationary phase and the micellar pseudophase. The separation occurs mainly on the basis of the differences in the polarities of the analytes, like in conventional RP-HPLC with aqueous–organic mobile phases.

In micellar electrokinetic capillary chromatog-

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raphy (MEKC) [1], the potential applied across a fused-silica capillary is the driving force of migration, like in capillary electrophoresis, CE. The difference between the two methods consists in using a running buffer containing an anionic surfactant (usually SDS) as an additive forming micelles at a concentration higher than the CMC. The electroosmotic flow (EOF) moves the running buffer (aqueous phase) in the capillary from the anode (injector) to the cathode (detector) at a velocity corresponding to the electroosmotic breakthrough time, t_{EOF}. The negatively charged micelles move in the same direction as the EOF, but at a lower migration velocity and eventually leave the capillary at the time t_{mic}. As the molecules of neutral analytes are distributed in between the micellar and the aqueous phases, all neutral analytes elute in the ''retention window'' between t_{EOF} and t_{mic}, and less polar solutes migrate to the detector more slowly than polar compounds as they spend more time in the micellar phase [2].

There are obvious principal similarities in the retention mechanism controlling aqueous–organic RP-HPLC, MLC and MEKC. Hence, it should be possible to correlate the retention in MEKC and the structure of the solutes in a similar way as in RP-HPLC. Linear solvation energy relationships (LSERs) have been suggested for characterisation of solvent-related properties of analytes (i.e., the logarithm of the retention factor, log k) by the linear combination of the solvatochromic parameters: molar volume of the analyte, dipolarity/polarisability interactions with the solvent and the analyte's basicity and acidity. As reported by Yang and Khaledi [3], LSER models for retention of 16 compounds in MLC and of 25 compounds in MEKC, respectively, 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 parameter of lipophility and the retention indices of various compounds [4,5].

The retention index concept, widely used in GC [6] and in HPLC [7], has been applied in MEKC by Muijselaar et al. [8] for the characterisation of neutral aromatic compounds and of the retention properties of pseudo-stationary phases in MEKC [9]. 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 the peak identification in MEKC.

Because of a limited retention window available for the elution of all neutral analytes in MEKC, the time interval decreases between the peaks with constant increments in partition ratios as the polarity of analytes decreases and the migration times become closer to t_{mic}, as it follows from Eq. (5) in the theoretical part. Hence the resolution in MEKC is often higher for more polar analytes than for the less polar ones. The retention window can be extended by reducing the electroosmotic flow [2]. To take full advantage of MEKC with reduced (ideally zero) electroosmotic flow (RF-MEKC) [10] for weakly polar compounds, the polarity of the potential applied on the capillary is reversed with respect to the conventional MEKC. Negative potential is applied to the injector end of the separation capillary (cathode) and the micelles of the anionic surfactant (SDS) move to the detector end of the capillary (anode) at a higher velocity than all non-ionic analytes. Like in conventional MEKC, the separation in RF-MEKC is based on the interactions between the micelles and the nonionic analytes. These interactions are enhanced for more lipophilic compounds, but opposite to the conventional MEKC, the retention of nonionic compounds increases with their increasing polarity and strongly polar solutes, which theoretically do not interact at all with the micelles, do not migrate along the capillary, as the aqueous phase does not move in the absence of electroosmotic flow. Hence, the most polar analytes are eluted first in MEKC, but last in RF-MEKC. Unlike MEKC, there is no upper limit of migration times and the resolution for low polarity analytes is enhanced, so that RF-MEKC is well suited for rapid separations of hydrophobic compounds, which may be poorly resolved or very strongly retained in conventional MEKC [11,12].

Chemical modification of the inner walls of fused-silica capillaries by creating a monomolecular polymeric layer largely suppresses the electroosmotic flow and undesirable adsorption of high-molecular-mass compounds on the capillary [13]. The principle of the wall modification consists in the chemical reaction with bifunctional reagents such as e.g., vinyl acetoxy silane, γ-methacryl propyl trimethoxy silane, vinyl trichlorosilane, methyl vinyl dichlorosilane, etc. One active reagent group reacts with the capillary wall and the other with a monomer forming eventually the polymer layer, such as polyacrylamide [14], methylcellulose [13], polyethylene glycol [14],

vinyl-polyacrylamide [15], polyvinylpyrrolidone [16,17], or octyl or octadecyl layers similar to the bonded phases used in RP-HPLC [18].

In the present work, we compare possibilities of calibration of the retention scale in MEKC and in RF-MEKC using the polar and the lipophilic indices, suggested earlier in RP-HPLC [19–21] and applied later in conventional MEKC [22].

The combination of the two indices in reversed-phase HPLC enables more precise prediction of the retention in mobile phases with different composition than a single retention index scale, as the two indices can better respect different contributions of the analytes and of the mobile phase to solvophobic and polar interactions and to the retention. It is assumed theoretically that the solvophobic effect is the main driving force in RP-HPLC, micellar HPLC, MEKC and RF-MEKC. Hence the retention calibration using the two-indices system may be potentially useful for the prediction of the retention in one mode from the data determined in another mode and for the transfer of actual analytical methods among various separation techniques.

2. Calibration of retention using polar and lipophilic indices

A simple method for characterisation of retention originally suggested for RP-HPLC is based on lipophilic and polar indices of solutes, n_{ce} and q_i [19–21]. The lipophilic index n_{ce} has the meaning of a hypothetical equivalent number of carbon atoms in the alkyl chain of a suitable calibration homologous series and increases with increasing lipophility of the analyte. It may depend on the type of the calibration series, but — ideally — it should not be significantly affected by the column packing material and by the type of organic solvent in the mobile phase. The index q_i is a measure of polar analyte–solvent interactions and is expected to depend strongly on the organic solvent and (possibly) on polar groups in the stationary phase, if any. The indices scale is based on a suitable calibration homologous series such as *n*-alkylbenzenes or alkan-2-ones, for which linear dependence of the logarithms of retention factors, *k*, on the volume fraction of the organic solvent, φ, in the binary mobile phase is assumed and the parameters *a* and *m* depend on the number of carbon atoms, i.e., of the repeat methylene units, *n*:

$$\log k = a - m\varphi = a_0 + a_1 n - (m_0 + m_1 n)\varphi$$
$$= (a_0 + a_1 n)(1 - p\varphi) - q\varphi \quad (1)$$

The parameters *a* (extrapolated logarithm of *k* in pure water) and *m* (contribution to the log *k* by the change from water to 100% organic solvent) in the calibration homologous series are correlated by linear relationship: $a = q + pm$. The correlation parameter $p = m_1/a_1$ is close to unity for homologous series with the repeat methylene unit. The parameter $q = a_0 m_1/a_1 - m_0$ is a combined function of the structure of the repeat methylene unit and of the end group in the calibration homologous series and depends on the mobile phase components. a_0 and a_1 characterise the contribution of the end group and of the homologous repeat unit, respectively, to log *k* in pure water; m_0 and m_1 characterise the effect of the change from water to 100% organic solvent on the contributions of the end group and of the repeat unit, respectively, to log *k*.

General validity of Eq. (1) for various analytes is assumed, as each compound is considered as a ''hypothetical'' member of the calibration homologous series, with its own lipophilic, n_{ce}, and polar, q_i, indices, as the equivalents of the parameters *n* and *q* [19]:

$$\log k = (a_0 - a_1 n_{ce})(1 - p\varphi) - q_i\varphi \quad (2)$$

To calculate the indices n_{ce} and q_i for various non-homologous sample compounds, parameters a_0, a_1 and *p* of the calibration homologous series are introduced into Eqs. (3) and (4), together with the constants *a* and *m* of Eq. (1) for each analyte [19]:

$$n_{ce} = \frac{a - a_0}{a_1} \quad (3)$$

$$q_t = m - p(a_0 - a_1 n_{ce}) \quad (4)$$

A similar approach can be used for the calibration of retention in MEKC, where the micellar pseudo-stationary phase theoretically has similar properties as the non-polar stationary phase in RP-HPLC [22]. In this case, the migration velocity of analytes depends on the concentration of the micelles in the running buffer, $c_{mic} = c_{surf} - \mathrm{CMC}$.

The retention factors in MEKC can be calculated from the migration times of analytes, t_R, if the electroosmotic breakthrough time, t_{EOF} and the migration time of micelles, t_{mic}, are known:

$$k = \frac{t_R - t_{EOF}}{t_{EOF}(1 - t_R/t_{mic})} \quad (5)$$

This equation can be rearranged to show that the difference in the migration times of two analytes with the same difference in retention factors decreases as the retention factors increase. This means that the resolution of more retained less polar compounds in MEKC impairs with respect to the resolution of the less retained more polar ones, contrary to isocratic HPLC or to GC where the difference in the retention times does not change for a constant difference in k.

From the experimental data, linear relationship between the logarithm of the retention factors, k, of analytes and the logarithm of the concentration of micelles in the running buffer, c_{mic}, was found:

$$\log k = a + m\log c_{mic} \quad (6)$$

The parameters a and m of Eq. (6) have similar meaning as the constants in Eq. (1), of course with respect to the concentration of micelles instead to the concentration of organic modifier.

The separation mechanism in MEKC is based on different partitioning between two moving phases, an electroosmotically driven aqueous phase and a micellar pseudophase. The partitioning of each analyte **S** is characterised by the ratio of its concentrations in the micellar, $[S]_{mic}$, and in the aqueous, $[S]_{aq}$, phases, i.e., by the partition coefficient of the analyte, K_D:

$$K_D = \frac{[S]_{mic}}{[S]_{aq}} \quad (7)$$

As non-polar analytes are distributed between the lipophilic micellar and the aqueous phases principally on the basis of their polarities, K_D is expected to increase with increasing lipophility of the analytes, much like RP-HPLC. The retention factor k in MEKC is a function of the concentration of micelles in the micellar running buffer:

$$k = K_D \cdot \frac{V_S}{V_M} = K_D \cdot \frac{\text{const}\, c_{mic}}{V_M} \quad (8)$$

where V_S is the volume of the micellar pseudophase, which is directly proportional to the concentration of the micelles, c_{mic}, and V_M is the volume of the aqueous phase in the separation capillary. Using the logarithmic form of Eq. (8), we obtain Eq. (6) with the parameter $m=1$:

$$\log k = a + \log c_{mic} \quad (9)$$

Using the same calibration approach as in RP-HPLC, the retention can be described as:

$$\log k = a + m\varphi = a_0 + a_1 n + (m_0 + m_1 n)\varphi$$
$$= (a_0 + a_1 n)(1 + p\varphi) + q\varphi \quad (10)$$

for the members of the homologous calibration series and

$$\log k = (a_0 + a_1 n_{ce})(1 + p\log c_{mic}) + q_i \log c_{mic} \quad (11)$$

for non-homologous compounds, where n_{ce} and q_i are the indices calculated in the same way as in RP-HPLC, using the retention data for the calibration series and for the analyte [22]. Other parameters have the same meaning as in Eq. (1).

Theoretically, the values of $q = q_i = m_0 = 1$ and $p = m_1 = 0$ are expected in MEKC, if the distribution of sample compounds between the aqueous phase and the micellar pseudo-stationary phase is controlled only by hydrophobic mechanism. The values of the parameter n_{ce} characterise the hydrophobicity of the solute and the values of the parameter q_i different from 1 indicate non-ideal behaviour, i.e. possible ionic interactions or contribution of electrophoretic migration of partly ionised compounds.

In RF-MEKC, the retention factor, k, can be calculated as in column chromatography provided the EOF can be neglected:

$$k = \frac{t_R - t_{mic}}{t_{mic}} \quad (12)$$

where t_R is the retention time of the analyte and t_{mic} is the retention time of the micelle migration marker, corresponding to the column hold-up time in HPLC or in GC. As in column chromatography, a constant difference in retention factors provides a constant difference in the retention times independent of the value of k, so that the resolution of lipophilic compounds is not impaired, in contrast to conventional MEKC. Moreover, the migration time order is

reversed with respect to the conventional MEKC and more lipophilic analytes exit from the capillary before the more polar ones and are often better separated, as the best resolution in the shortest time is obtained at k close to 2.

The retention factors of the analytes in MEKC calculated from Eq. (5) cannot be compared directly with the k in RF-MEKC calculated from Eq. (12). The reason is that in conventional MEKC, $k=0$ for very polar compounds with migration times equal to the breakthrough time of the electroosmotic flow, t_{EOF}, but in RF-MEKC, $k=0$ for the analytes with migration times equal to the migration times of micelles, i.e., for very lipophilic compounds and the order of elution is opposite in the two techniques, like in RP-HPLC with respect to normal-phase HPLC. Hence, the retention factors in the two techniques determined using Eqs. (5) and (12) cannot be compared directly. That is why we used reciprocal values of the retention factors in RF-MEKC, $1/k$, for comparison with k in MEKC and in RP-HPLC, as these values increase with lipophility of the analytes. In agreement with this concept, we found linear relationship between $1/k$ and the logarithm of the concentration of micelles in the running buffer, c_{mic}:

$$\log (1/k) = a + m \log c_{mic} \quad (13)$$

Using a similar approach for the calibration of retention as in MEKC, the retention of non-homologous solutes in RF-MEKC can be described by Eq. (14):

$$\log (1/k) = (a_0 + a_1 n_{ce})(1 + p \log c_{mic}) + q_i \log c_{mic} \quad (14)$$

with the same meaning of the values of a_0, a_1, q_i, m_0, p and m_1 as in conventional MEKC if the partition between the phases is controlled only by solvophobic mechanism.

3. Experimental

The standards of phenylurea and triazine herbicides were obtained from Synthesia, Pardubice-Semtín. Czech Republic. Acetone, ethylmethyl ketone, *N*-propylmethyl ketone, *N*-butylmethyl ketone and *N*-hexylmethyl ketone, ethylbenzene, *N*-propylbenzene, (3-methacryloyloxypropyl)trimethoxysilane, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and SDS (all analytical grade) were obtained from Fluka, Buchs, Switzerland. Sodium tetraborate, boric acid, sodium hydrogenphosphate, sodium dihydrogenphosphate, potassium persulphate, benzene and toluene (all analytical grade) and methanol (UV grade) were obtained from Lachema, Brno, Czech Republic. Acyrlamide (99+%) was purchased from Aldrich, Prague, Czech Republic.

A Crystal 310 capillary zone electrophoresis instrument (ATI Unicam, Cambridge, UK) equipped with a variable-wavelength UV detector was used. The conventional MEKC experiments were performed in a fused-silica capillary, 75 cm (60 cm effective length to the detector) 50 μm I.D. (J&W, Folsom, US). Mixtures of 0.025 *M* borate buffer (pH 8.5) with appropriate addition of SDS were used as the running buffers. The capillary was subsequently washed with 0.1 *M* NaOH (10 min), water (10 min) and with the running buffer (until a stabilised baseline was obtained) before use. MEKC separations were performed at a potential of +20 kV applied across the capillary.

For the RF-MEKC experiments, a capillary with the internal walls modified with polyacrylamide coating was prepared. A two-stage coating method (silanisation of the capillary pretreated with NaOH followed by bonding and polymerisation of polyacrylamide) was used as described by Cobb et al. [14]. The dimensions of the capillary were the same as in the MEKC experiments. Mixtures of 0.025 *M* phosphate buffer (pH 6.0) with appropriate addition of SDS were used as the running buffers. Before use, the modified capillary was subsequently washed with water for 2 h and then with the running buffer until a stabilised baseline was obtained. RF-MEKC separations were performed at a potential of −17.5 kV.

The temperature of the capillary was set at 35°C in both MEKC and RF-MEKC experiments. The detector was operated at 230 nm, except for the detection of ketones (270 nm). Methanol (in MEKC) or acetone (in RF-MEKC) were used as the electroosmotic flow time, t_{EOF}, markers and Sudan II (Lachema, Brno) was used as the micelle migration time, t_{mic}, marker. The retention factors of analytes were calculated from the migration times, t_R, in

MEKC using Eq. (5) and in RF-MEKC using Eq. (12). Eq. (12) is valid under the ideal separation conditions only, where zero electroosmotic flow is assumed. For the data obtained with a non-zero electroosmotic flow in the capillaries, Eq. (15) formally similar to Eq. (5) was employed, which was derived from Eq. (12) with appropriate correction to account for this phenomenon:

$$k = \frac{t_R - t_{mic}}{t_{mic}(1 - t_R/t_{EOF})} \quad (15)$$

4. Results and discussion

4.1. Electroosmotic flow determination

The residual electroosmotic flow in modified capillaries was too low to be measured directly. Hence it was determined from the following set of experiments: (i) In the first experiment, a neutral EOF marker (acetone) was pushed through the capillary at a small overpressure (100 mbar) applied to the injector end of the separation capillary and the ''migration time'' of the zone of acetone caused only by the overpressure was registered. (ii) In the second experiment, a high voltage (+17.5 kV) was applied for a defined time period of 10 min from the introduction of acetone into the capillary. After the end of this period, the same overpressure (100 mbar) was applied until the acetone peak was registered, so that acetone migrated a certain distance along the capillary by virtue of overpressure only and the rest of the distance by electroosmotic flow. (iii) The second experiment was repeated for other time periods of the application of high voltage to the capillary (15, 20 and 30 min). (iv) The experimental migration times of acetonitrile during each overpressurised migration period were used to calculate the part of the capillary length corresponding to the EOF migration in subsequent experiments. These lengths were plotted dependent on the time interval of the applied high voltage and the t_{EOF}, corresponding to the full effective capillary length (60 cm) extrapolated from the linear plot.

The EOF times for the two modified capillaries prepared were 250 min and 223 min, respectively; this means that the EOF was reduced to less than 4% in comparison with unmodified capillaries. The error of the determination of t_{EOF} was approximately 2%.

4.2. Retention of homologous series in MEKC and RF-MEKC

In MEKC, the experimental logarithms of the retention factors of the calibration homologous *n*-alkan-2-one standards increase in linear manner as the logarithm of concentration of micelles of SDS in working electrolytes increases, in agreement with Eq. (6) and as the number of carbon atoms, *n*, increases, in accordance with earlier results in aqueous–organic RP-HPLC and in MLC [22] (Fig. 1). The experimental values of the slopes, *m,* of Eq. (6) were close to one in the MEKC experiments, in agreement with Eq. (9) and the agreement improved for the standards with longer alkyl chains.

In RF-MEKC, two calibration homologous series were used: *n*-alkan-2-ones and *n*-alkylbenzenes. The reciprocal values of the retention factors must be used for the description of retention to obtain comparable results with MEKC, micellar HPLC and RP-HPLC, as discussed above. The same conclusions as in MEKC apply to the dependence of log $(1/k)$ versus log c_{mic} and for the dependence of log $(1/k)$ versus *n*. The values of *k* were calculated both from Eq. (12) for the ideal separation conditions assuming zero electroosmotic flow and from Eq. (15) for real separation conditions with residual electroosmotic flow determined experimentally as described above (Fig. 2).

Table 1 summarises the values of the parameters a_0, a_1, m_0, m_1, p and q of Eq. (10) in the MEKC and RF-MEKC separation systems studied. The parameters m_0 and q are close to one, as well as the parameters m_1 and p are close to zero, except for the values of the parameters in the RF-MEKC system calculated using Eq. (5) for non-zero electroosmotic flow with the ketone calibration series. The plots in Fig. 2 and the values of the parameters in Table 1 show that the errors introduced by neglecting the residual EOF are minor if the homologous series of *n*-alkylbenzenes is used for calibration, but are more significant with the homologous *n*-alkan-2-ones calibration series. It should be noted that in aqueous–organic RP-HPLC of homologous series the values of m_1 and p are always positive. Negative values in

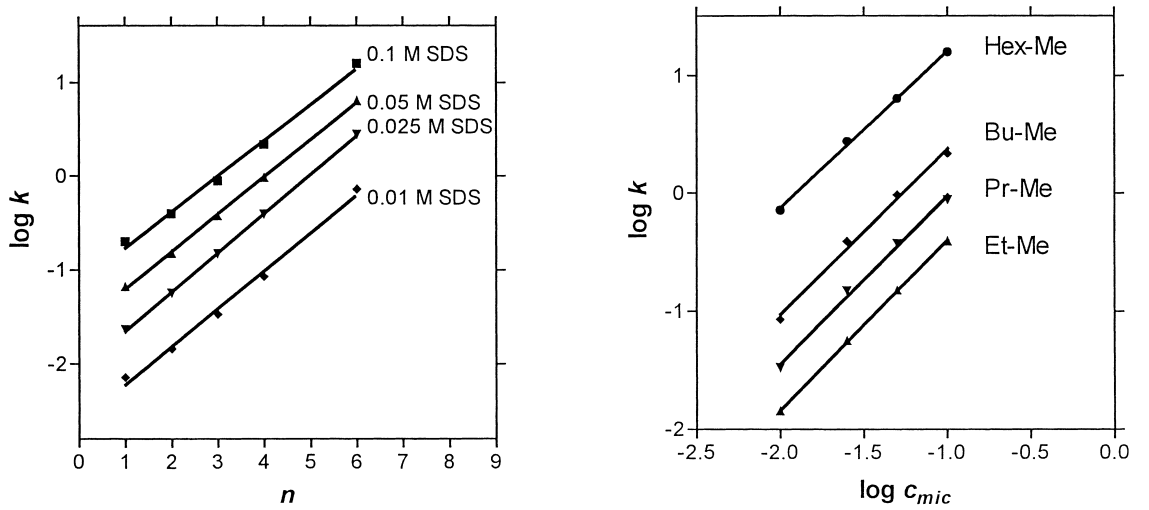

Fig. 1. Dependencies of log k on the logarithm of micelle concentration, c_{mic}, of sodium dodecylsulphate [SDS, Eq. (5)] and dependencies of log k on the number of carbon atoms in the alkyl chain for the homologous n-alkane-2-one calibration series in MEKC (Me = methyl, Et = ethyl, Pr = n-propyl, Bu = n-butyl, Hex = n-hexyl).

RF-MEKC are caused by decreasing retention in RF-MEKC with increasing lipophility of the analytes, opposite to the behaviour in RP-HPLC and in conventional MEKC.

Fig. 3 shows the dependence of a versus n for the n-alkylbenzene homologous calibration series in RF-MEKC and for the n-alkan-2-one homologous calibration series in MEKC separation systems. In both systems, strong linear correlation between the two parameters was observed, in agreement with the behaviour in RP-HPLC observed earlier [19–21]. This justifies the assumption of reversed-phase (solvophobic) effect as the main factor controlling the distribution of the calibration homologous com-

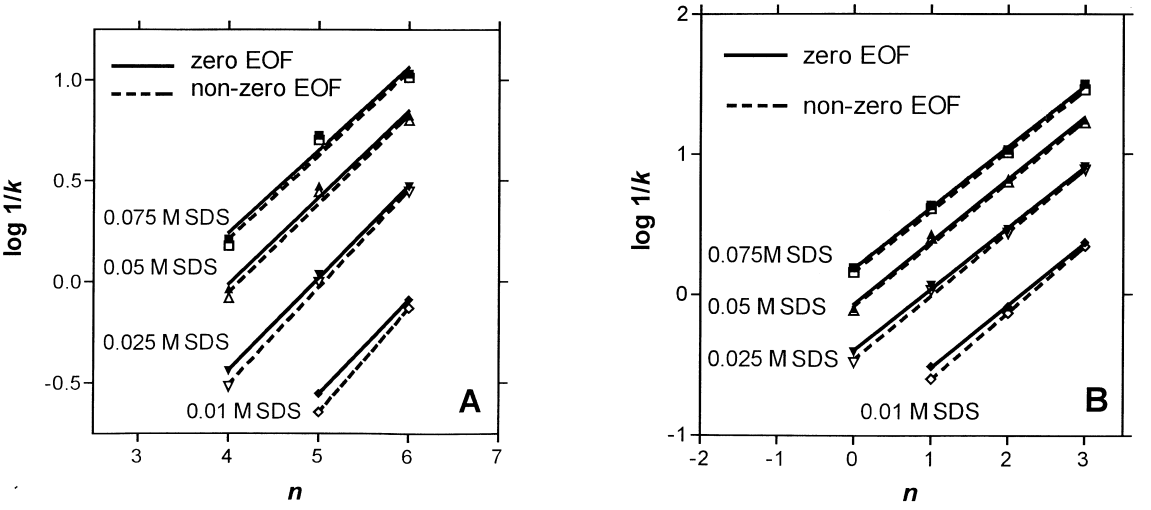

Fig. 2. Dependencies of log k on the number of carbon atoms, n, in the alkyl chains for different calibration series in RF-MEKC. Homologous n-alkan-2-one (A) and homologous n-alkylbenzene (B) calibration series. SDS = sodium dodecylsulphate.

Table 1
Parameters a_0, a_1, m_0 m_1 p and q of Eq. (10) of the calibration homologous series of *n*-alkan-2-ones for MEKC and RF-MEKC. R=Correlation coefficent. Log k values were used in MEKC [Eq. (11)] and log $(1/k)$ values in RF-MEKC [Eq. (14)] for determination of the parameters

System	Parameters						
	a_0	a_1	R	m_0	m_1	p	q
MEKC (ketones)	−0.047	0.337	0.994	1.163	−0.027	0.063	1.149
RF-MEKC (ketones, zero EOF)	0.190	0.329	0.821	1.359	−0.071	−0.087	1.163
RF-MEKC (ketones, non-zero EOF)	0.383	0.294	0.812	1.562	−0.105	−0.201	1.411
RF-MEKC (Benzenes, zero EOF)	1.271	0.418	0.998	0.923	−0.009	−0.021	0.950
RF-MECK (benzenes, non-zero EOF)	1.306	0.397	0.991	0.930	−0.009	−0.073	1.703

pounds between the aqueous and the micellar phases, both in conventional MEKC and in RF-MEKC. Further, Fig. 3B illustrates relatively non-significant error introduced by the neglection of the residual EOF in the description of migration of *n*-alkylbenzenes.

4.3. Calibration of the retention of the tested compounds

Good linearity was found for the plots of log k [Eq. (6)] and of log $(1/k)$ [Eq. (13)], respectively, of the analytes tested versus the logarithm of con-

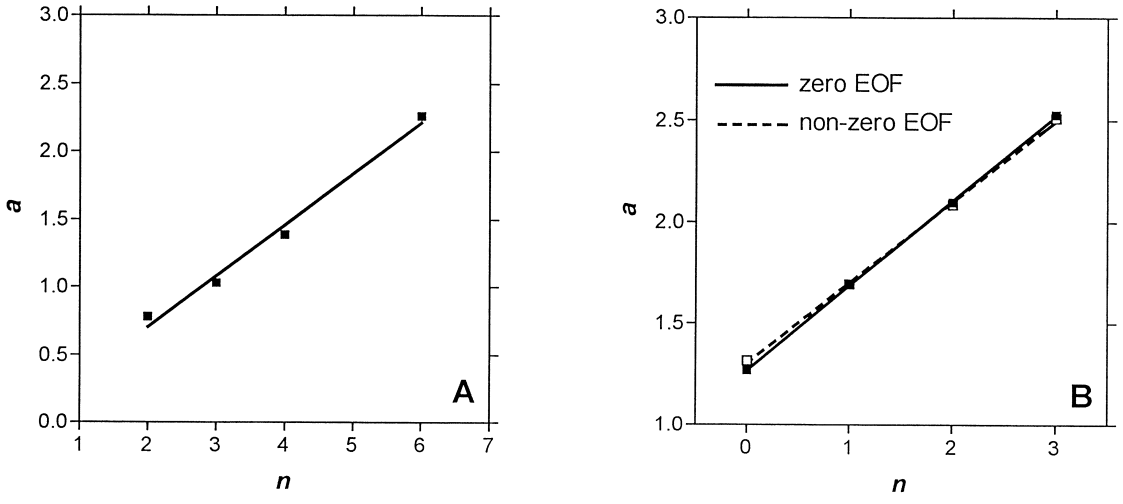

Fig. 3. Dependencies of the parameter a, versus the number of carbon atoms, n, in the alkyl chains in MEKC and RF-MEKC. Homologous *n*-alkane-2-one calibration series in MEKC (A) and homologous *n*-alkylbenzene calibration series in RF-MEKC (B).

Table 2
Calculated lipophilic and polar indices, n_{ce} and q_i, of some phenylurea and triazine herbicides and barbiturates used as test compounds in MEKC and RF-MEKC systems with running buffers containing SDS and in RP-HPLC systems with a Silasorb C_8 column and water–methanolic mobile phases. *n*-alkan-2-ones were used as the calibration series

Compound	MEKC, conc. of SDS 0.025–0.1 *M*		RF-MEKC, conc. of SDS 0.01–0.075 *M*		RP-HPLC conc. of methanol 25–50%	
	n_{ce}	q_i	n_{ce}	q_i	n_{ce}	q_i
Phenuron	**3.37**	1.04	**2.93**	1.04	**3.33**	1.85
Desphenuron	**3.48**	1.02	**3.10**	1.04	**2.93**	1.58
Deschlorometoxuron	**3.80**	1.01	**3.33**	1.04	**3.83**	2.25
Metoxuron	**4.94**	1.03			**4.87**	2.61
N-Phenylurea	**3.35**	1.04	**3.18**	0.96	**2.76**	1.88
Linuron	**6.66**	1.10	**6.48**	1.05	**6.62**	1.91
Simazine	**5.27**	1.05	**5.13**	1.80	**4.96**	1.97
Atrazine	**6.13**	1.09	**5.46**	0.95	**5.68**	1.85
Phenobarbital	0.99	0.19	3.27	0.93		
Cyclobarbital	1.56	0.36	3.84	0.98		
Ammobarbital	2.83	0.60	4.38	0.92		
Pentobarbital	3.79	0.79	4.90	1.01		
Hexobarbital	3.88	0.86	4.10	0.92		

centration of SDS micelles. The values of the slope, *m*, of these equations are close to one, as expected, for the phenylurea and triazine herbicides both in MEKC and in RF-MEKC. In MEKC, the values of the parameters, *m*, of barbiturates were significantly lower than one because of their weak acidic character. This phenomenon was not found in the RF-MEKC system, where their ionisation is suppressed in the running buffer with a lower pH. In MEKC, pH 8.5 had to be used to achieve sufficient EOF for adequate migration times in uncoated capillaries. In RF-MEKC, pH 6 was used as there was no EOF limitation and also because the polymeric layer in modified capillaries was not stable enough against the hydrolysis at a pH higher than 7.

The lower homologous *n*-alkan-2-ones are more suitable standards than *n*-alkylbenzenes for the calibration of the two-index retention scale for conventional MEKC, as their migration times correspond better to more polar analytes, for which the MEKC technique is more suitable than RF-MEKC. On the contrary, less polar *n*-alkylbenzenes are more suitable as the retention calibration standards for RF-

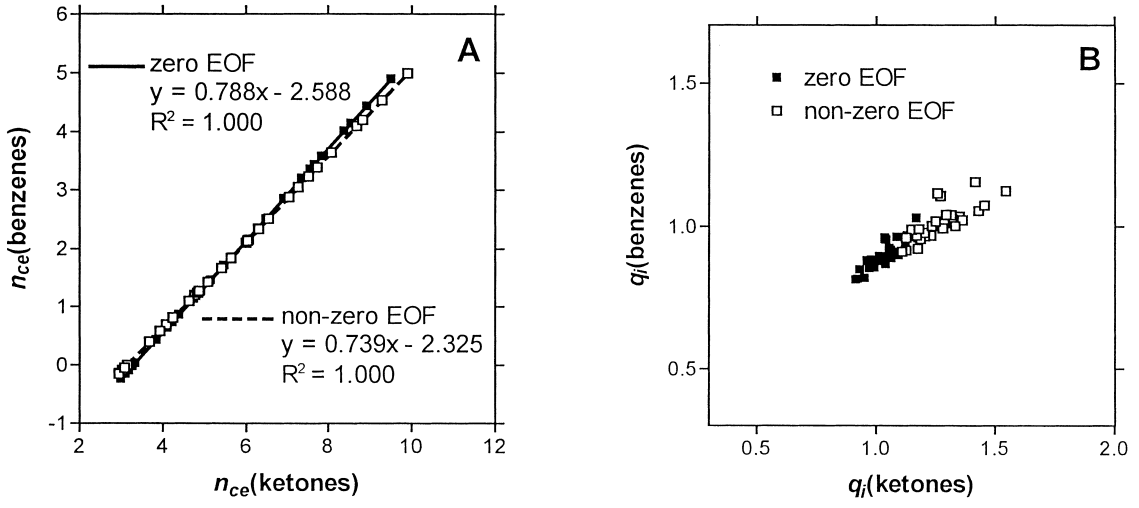

Fig. 4. Correlation between the lipophilic indices n_{ce} (A) and the polar indices q_i (B) of tested compounds determined using two different calibration series in RF-MEKC.

MEKC, which is more advantageous for separations of less polar analytes than conventional MEKC.

The parameters q_i determined using Eqs. (11) and (13) are close to one for all analytes tested, except for barbiturates in the MEKC system (Table 2). Lower values of q_i for these compounds, which can be partially ionised at pH>7, can be probably attributed to the contribution of the electrophoretic mobility to the migration and may be used as an indicator of such non-ideal behaviour.

The linear correlation between the parameters n_{ce} and the correlation between the parameters q_i of tested compounds calculated using the calibration homologous series of *n*-alkylbenzenes and of *n*-alkan-2-ones in RF-MEKC are shown in Fig. 4. The points correspond to 23 compounds tested. The correlations between the lipophilic parameters n_{ce} is very good, even if the residual EOF is neglected. Theoretically, all the points in the q_i(*benzenes*) *v*ersus the q_i(*ketones*) plots should have values close to the coordinates (1.0, 1.0), but the experimental points are slightly shifted from this theoretical coincidence point and the values of the polar indices depend on whether the EOF is neglected or not in the determination of *k*.

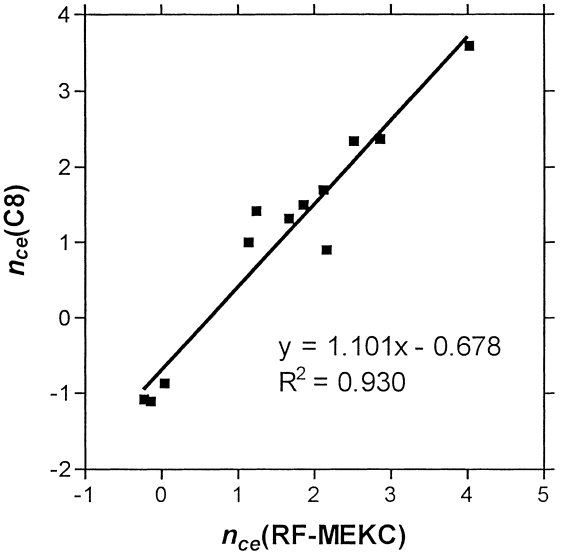

Fig. 5. Correlation between the lipophilic indices n_{ce} determined in RF-MEKC and in RP-HPLC with a Silasorb C_8 octyl-silica column and methanol–water mobile phases.

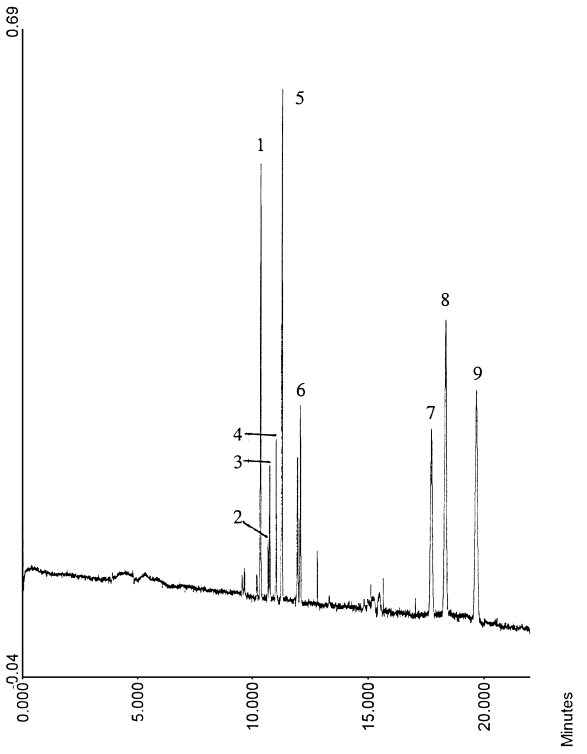

Fig. 6. RF-MEKC separation of a mixture of phenylurea herbicides. Capillary, polyacrylamide coated fused-silica 75 cm (60 cm effective length), 50 μm I.D. Running buffer: 0.075 *M* SDS in 0.025 *M* phosphate buffer (pH 6). Voltage, −17.5 kV. Analytes: 1=neburon, 2=isoproturon, 3=diuron, 4=*N′*-butyl-*N*-phenylurea, 5=monolinuron, 6=monuron, 7=desphenuron, 8=phenuron, 9=*N*-phenylurea.

Table 2 compares the values of the lipophilic and of the polar indices calculated for MEKC and RF-MEKC systems with those obtained in a RP-HPLC system with an octyl silica column in aqueous–methanolic mobile phases. The values of the indices n_{ce} are close to each other in all systems, except for phenylurea and desphenuron (possibly because of higher polarities of these two compounds). The correlation between the n_{ce} values in RF-MEKC and octyl-silica RP-HPLC systems is illustrated in Fig. 5.

Figs. 6 and 7 compare the separation of some herbicides in MEKC and in RF-MEKC. The order of elution in the two systems is reversed and RF-MEKC enables the separation of some less polar herbicides, such as isoproturon and diuron, which was not

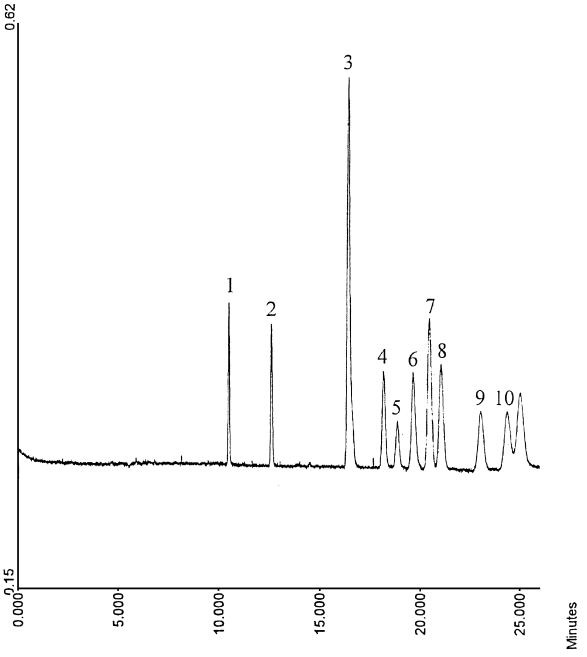

Fig. 7. MEKC separation of a mixture of phenylurea herbicides. Capillary, uncoated fused-silica 75 cm (60 cm effective length× 50 μm I.D. Running buffer: 0.1 *M* SDS in 0.025 *M* borate buffer (pH 8.5). Voltage, +20 kV. Analytes: 1=*N*-phenylurea, 2= deschlorometoxuron, 3=monuron, 4=monolinuron, 5= fluometuron, 6=*N′*-butyl-*N*-phenylurea, 7=chlorotoluron, 8= isoproturon, 9=chlorobromuron, 10=neburon.

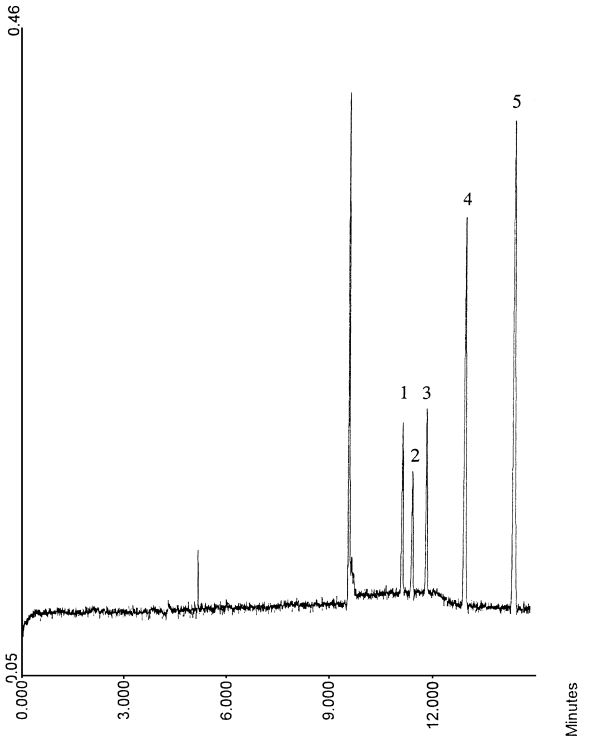

Fig. 8. RF-MEKC separation of a mixture of barbiturates. Capillary, polyacrylamide coated fused-silica 75 cm (60 cm effective length×50 μm I.D. Running buffer: 0.075 *M* SDS in 0.025 *M* phosphate buffer (pH 6). Voltage: −17.5 kV. Analytes: 1= pentobarbital, 2=amobarbital, 3=hexobarbital, 4=cyclobarbital, 5=phenobarbital.

accomplished in conventional MEKC. The separation of barbiturates shown in Fig. 8 was not feasible in conventional MEKC, where the differences in the retention factors of the individual analytes were too low.

5. Conclusions

Retention behaviour of various barbiturates, phenylurea and triazine herbicides was compared in MEKC with uncoated fused-silica capillaries and in RF-MEKC using capillaries modified with linear polyacrylamide, where the EOF was reduced to less than 4% of the EOF in uncoated capillaries.

A method was suggested for the determination of low residual EOF in wall-coated capillaries. Even though the coating process reduced the EOF to approximately 4% of the original value, its neglection may affect the retention data and the values of the indices characterising the retention.

The retention was characterised using the lipophilic and polar indices introduced earlier to characterise the retention in RP-HPLC and in MLC. Homologous series of *n*-alkylbenzenes and of *n*-alkan-2-ones were compared as the standard sets for the calibration of the retention (migration) scale. The first series was found more suitable for RF-MEKC and the second for conventional MEKC. The values of the lipophilic indices of an analyte measured in various experimental techniques are close to each other in all systems discussed, except for phenylurea and desphenuron. The values of the polar indices significantly lower than one indicate an important contribution of the electrophoretic mobility of ionisable analytes to their migration.

Acknowledgements

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