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Equation for the description of the resolution of charged solutes in micellar electrokinetic capillary chromatography

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Abstract

The migration behaviour of charged solutes in micellar electrokinetic capillary chromatography is affected by both the electrophoretic properties of the solute and its interaction with the micelles. Both must therefore be considered to obtain an adequate description of the migration behaviour and thus resolution. A novel equation is presented, in which the resolution is expressed as a function of the micellar interaction (capacity factor for charged solutes) and the electrophoretic mobility (free zone migration time) of the charged solute. Simulations reveal the dependence of the resolution of two closely migrating solutes on both distinct interactions, which is clearly different as compared to the separation of neutral solutes and general guidelines with respect to better separation are deduced. Experimental data illustrate that this equation can be used to gain more insight in the corresponding migration mechanisms. Furthermore, using this knowledge, it is possible to adjust the experimental parameters to achieve better resolution.

Keywords: Resolution equation; Electrophoretic mobility; Micellar interaction; Migration behaviour; Capacity factors. micellar electrokinetic capillary chromatography

1. Introduction

Micellar electrokinetic capillary chromatography (MECC) is a special mode of capillary electrophoresis (CE) initially developed to separate neutral solutes [1–4]. The electroosmotic and the micellar migration velocities are driven by a potential difference across the capillary. The migration behaviour of a neutral solute then results from its partitioning between the aqueous and the micellar phases.

Selectivity between solutes therefore originates from differences in their interaction with the micelles. Such a separation mechanism is of course analogous to that observed in conventional chromatography and therefore, a capacity factor k was introduced to describe the interaction between the solute and the micellar phase [1]

$$k = \frac{t_m - t_{eo}}{t_{eo}\left(1 - \frac{t_m}{t_{mc}}\right)} \quad (1)$$

where t_m, t_{eo} and t_{mc} are the migration times of the solute, the electroosmotic flow and the micellar phase, respectively.

In practice, many samples also contain charged solutes and it has been shown that MECC is suitable to separate such mixtures as well [4–7], so that the

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number of relevant parameters which influence the migration is extended.

The migration behaviour of charged solutes is related to both the electrophoretic properties of the solute and its interaction with the micelle. The velocity in the aqueous phase is defined by the charge density of the solute but when the solute is associated with the micellar phase, its velocity is mainly determined by the micellar mobility. Hydrophobic forces play a major role when the solute interacts with the core of the surfactant aggregate and electrostatic attraction (or repulsion) plays a dominant role if prominent charged sites are present on both the solute and the micelle [8].

Otsuka et al. introduced a modified capacity factor to describe the micellar interaction of a charged solute [9]

$$k' = \frac{t_m - t_{m0}}{t_{m0}\left(1 - \frac{t_m}{t_{mc}}\right)} \tag{2}$$

where t_{m0} is the migration time of the solute in the absence of micelles. Note that k is used in the case of uncharged solutes, while k' pertains to charged solutes. The capacity factor as defined in Eq. (2) is analogous to the capacity factor for neutral solutes and is only a measure for the interaction between the solute and the micelle. However, the overall migration velocity is not only determined by the micellar interaction, quantitatively expressed as k', but also by the charge density of the solute and this results in an electrophoretic mobility in the aqueous phase different from the electroosmotic mobility. As such, translation of capacity factor values to the observed migration behaviour and subsequent resolution of two (closely migrating) solutes is not obvious.

Here, we present an equation describing the resolution as a function of capacity factors for charged solutes. The information comprised in k' values is translated into easily interpretable information coupled to the electropherogram of interest. By means of simulations, the influence of different parameters on the resolution is studied and using experimental data, it is shown that this equation is useful for a better understanding of the migration principles and in the search for good separation conditions.

2. Theory

2.1. A resolution equation for charged solutes in MECC

The mole fraction X of a solute in the aqueous phase is defined and related to the capacity factor k' as

$$X = \frac{n_{aq}}{n_{aq} + n_{mc}} = \frac{1}{1 + k'} \qquad \text{with } k' = \frac{n_{mc}}{n_{aq}} \tag{3}$$

where n_{aq} and n_{mc} are the number of moles in the aqueous and micellar phase respectively. The overall velocity of a solute v_r can then be expressed as a weighted sum of the velocity of the solutes in the absence of micelles v_0 and the velocity of the micellar phase v_{mc}

$$v_r = Xv_0 + (1 - X)v_{mc} \tag{4}$$

It follows that

$$X = \frac{v_r - v_{mc}}{v_0 - v_{mc}} = \frac{t_{mc} - t_m}{t_m} \frac{t_{m0}}{t_{mc} - t_{m0}}$$

$$\text{with } v = \frac{l}{t} \tag{5}$$

where l is the migration distance. Combination of Eqs. (3,5) results in

$$k' = \frac{1}{X} - 1 = \frac{t_m - t_{m0}}{t_{m0}\left(1 - \frac{t_m}{t_{mc}}\right)} \tag{6}$$

so that the migration time of a solute in a micellar system can be expressed as a function of the capacity factor, the migration time in the absence of micelles and the micellar migration time (see also [1,9,10]):

$$t_m = \frac{t_{m0}(1 + k')}{1 + \frac{t_{m0}}{t_{mc}}k'} \tag{7}$$

The resolution R_s between two solutes i and j is defined as

$$R_s = \frac{t_{mj} - t_{mi}}{4\sigma_j} = \frac{\sqrt{N}}{4}\left(1 - \frac{t_{mi}}{t_{mj}}\right) \tag{8}$$

where σ is the standard deviation of the peak and N the efficiency $(t_{mj}/\sigma_j)^2$. Substitution of Eq. (7) in Eq.

(8) results in the resolution equation applicable to samples containing charged solutes:

$$R_s = \frac{\sqrt{N}}{4} \frac{t_{m0j}(1+k'_j) - t_{m0i}(1+k'_i) + \frac{t_{m0i}t_{m0j}}{t_{mc}}(k'_i - k'_j)}{t_{m0j}(1+k'_j)\left(1+\frac{t_{m0i}}{t_{mc}}k'_i\right)} \quad (9)$$

This equation relates the resolution to the capacity factors and t_{m0} values, which are solute specific parameters. Here it is assumed that the electrophoretic mobility of the solute is constant and not influenced by the addition of SDS. All solute specific changes in the migration behaviour, like interactions with the micellar phase, as well as interactions with the monomers surfactant molecules and shifts of the dissociation constants of weak acids and bases due to the presence of the surfactant molecules, are lumped in one capacity factor.

To calculate the capacity factor according to Eq. (6), data obtained from two experiments need to be combined: a free zone experiment to measure t_{m0} and a micellar experiment to measure t_m and t_{mc}. The relevance of such a capacity factor is, however, dependent on the validity of the following three assumptions:

(I) The electroosmotic flow should not change significantly when going from a free zone to a micellar system. When significant changes occur, corrections must be included to adjust the t_{m0} values according to the electroosmotic mobility measured in the micellar system. In this way, the k' values are unaffected by changes in the observed electroosmotic flow.

From simple electrophoretic principles it follows that the observed apparent mobility of a solute in a free zone system ($\mu_{app\ CZE}$) equals the sum of the electroosmotic ($\mu_{eo\ CZE}$) and the electrophoretic mobility of the solute (μ_{ep}):

$$\mu_{app\ CZE} = \mu_{eo\ CZE} + \mu_{ep} \quad (10)$$

If the electroosmotic flow in the micellar experiment ($\mu_{eo\ MECC}$) is different from that in the free zone system, the apparent mobility (μ'_{app}) corresponding to the desired t_{m0} value can easily be calculated using the electroosmotic mobility measured in the micellar system.

$$\mu'_{app} = \mu_{eo\ MECC} + \mu_{ep} \quad (11)$$

Combination of Eqs. (10,11) results in

$$\mu'_{app} = \mu_{eo\ MECC} + \mu_{app\ CZE} - \mu_{eo\ CZE} \quad (12)$$

so that t_{m0} values, necessary to calculate capacity factors, can always be calculated.

(II) The kinetics of mass transfer between the aqueous and the micellar phase should be fast so that a solute is moving either in the aqueous phase or in the micellar phase and the intermediate state does not significantly contribute to the migration behaviour.

(III) The micellar mobility should be unaffected by the incorporation of a solute molecule. The validity of this assumption is related to the type of surfactant and solute and the actual micellar mobility depends on the net charge and the mass of both species. SDS, which is used throughout the experimental part of this study has a molecular weight of 288, carries one negative charge and has an aggregation number of 62 while the test solutes have molecular weights less than 200 and carrying at most one charge. It is therefore unlikely that both the charge or the size of SDS micelles are noticeably affected by the incorporation of one solute molecule. Similarly, the same assumption is made if the micellar mobility is measured using a hydrophobic marker like Sudan III, which is assumed to be totally solubilised in the micellar phase.

2.2. Evaluation of the resolution as a function of different parameters

The examples chosen here assume that the electroosmotic and the solute migration time are smaller than the micellar migration time. The interaction with the micelles always results in a slower migration velocity as compared to the free zone system. These assumptions are in accordance with the experimental conditions most frequently found in the literature. The equations presented, however, are not restricted in any way and are applicable for all possible values of t_{eo}, t_m and t_{mc}.

2.2.1. Simplification to the resolution equation for uncharged solutes

The resolution equation, as expressed in Eq. (9), is applicable to samples containing both charged and uncharged solutes. In case of uncharged solutes, t_{m0i}

and t_{m0j} equal t_{eo} and Eq. (9) reduces to Eq. (13) which is the well-known resolution equation for uncharged solutes in MECC derived by Terabe et al. [10].

$$R_s = \frac{\sqrt{N}}{4}\frac{(k_j - k_i) + \frac{t_{eo}}{t_{mc}}(k_i - k_j)}{(1+k_j)(1+\frac{t_{eo}}{t_{mc}}k_i)} = \frac{\sqrt{N}}{4}\frac{\alpha - 1}{\alpha}\frac{k_j}{1+k_j}\frac{1-\frac{t_{eo}}{t_{mc}}}{1+\frac{t_{eo}}{t_{mc}}k_i} \qquad t_{m0i} = t_{m0j} = t_{eo} \tag{13}$$

where α is the selectivity, defined as the ratio of the two capacity factors. The selectivity between two solutes is only dependent on the differences in the micellar interaction and thus their capacity factor values. This is clearly different when compared to the equation for charged solutes (Eq. (9)) where both the micellar interaction as well as the electrophoretic properties of the solutes must be taken into account.

2.2.2. The resolution as a function of k'_i and k'_j

In the resolution equation for uncharged solutes (Eq. (13)), the capacity factors are the only solute specific parameters and maximisation of the resolution can be performed by adjusting the capacity factors. This was illustrated by Terabe et al. [10] who evaluated the effect of k on the resolution. Assuming both the efficiency and the selectivity of neutral solutes being independent of the capacity factor, Eq. (13) was simplified to yield the f(k) function as shown in Eq. (14).

$$\mathrm{f}(k) = \frac{k_j}{1+k_j}\frac{1-\frac{t_{eo}}{t_{mc}}}{1+\frac{t_{eo}}{t_{mc}}k_i} \approx \frac{k}{1+k}\frac{1-\frac{t_{eo}}{t_{mc}}}{1+\frac{t_{eo}}{t_{mc}}k} \qquad k_i \approx k_j \approx k \tag{14}$$

The k value which corresponds to the maximum of this function depends on the ratio of t_{eo}/t_{mc}. It was concluded in this study [10] that intermediate values of k (2 to 5) would result in optimal separation conditions. This is in agreement with the results obtained from other studies in which the resolution of neutral solutes in MECC was investigated [11,12].

If the assumption $k_i \approx k_j$ is omitted, both capacity factors must be considered to evaluate the resolution. In this work, the complete resolution equation for uncharged solutes (Eq. (13)) is used to evaluate R_s as a function of k_i and k_j, and only N is assumed to be independent of the capacity factor. This is shown in the iso-resolution plot in Fig. 1 which is characterised by a high degree of symmetry, the diagonal from low to high k values being the symmetry axis. On this diagonal, k_i and k_j are equal and consequently the resolution is always zero.

At low k values, the term $k_j/(1+k_j)$ will be small and dominates the R_s value. At high k values, both solutes will migrate close to the t_{mc} and the separation will be poor due to the last term in Eq. (13). At intermediate values of k, only relatively small differences between k_i and k_j are required to obtain a significant increase of the resolution and this range of k values should be preferred, in agreement with the aforementioned studies. Furthermore, maximisation of the difference between k_i and k_j always results in an increase in the observed resolution.

If charged solutes are present, the micellar interaction can be quantified using the appropriate capacity factor for charged solutes but then the resolution equation for charged solutes (Eq. (9)) must be used

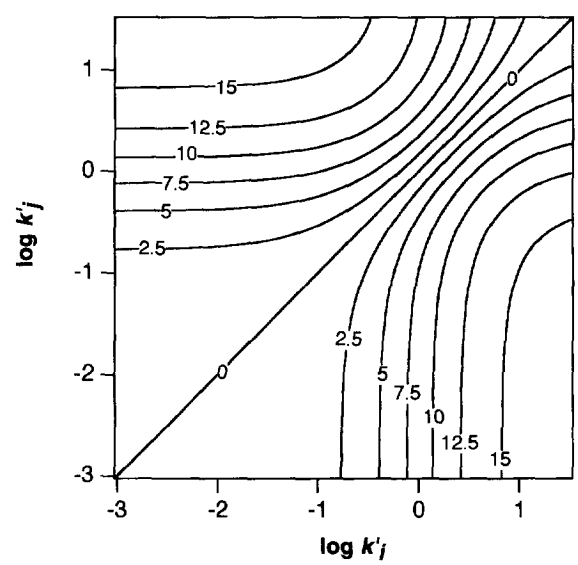

Fig. 1. Iso-resolution plot as a function of k_i and k_j (logarithmic scale) for two uncharged solutes (Eq. (13)). The value of N is set to 10 000, t_{eo} and t_{mc} are set to 3.1 and 10, respectively.

to evaluate the quality of the separation and to estimate k' values resulting in adequate resolution. Three situations are distinguished:

2.2.3. Case 1: $t_{m0i} = t_{m0j} = t_{m0}$

The electrophoretic properties of both solutes are equal and simplification of Eq. (9) to an equation analogous to the one for uncharged solutes results in:

$$R_s = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k'_j}{1 + k'_j} \frac{1 - \frac{t_{m0}}{t_{mc}}}{1 + \frac{t_{m0}}{t_{mc}} k'_i}$$

$$t_{m0i} = t_{m0j} = t_{m0} \qquad (15)$$

An analogous equation describing the resolution for this particular case has already been presented [13].

It is clear that the iso-resolution surface as a function of the capacity factors is similar to that for uncharged solutes as illustrated in Fig. 2. However, the absolute values of the resolutions are smaller in the case of $t_{eo} < t_{m0}$ and larger in the case of $t_{eo} > t_{m0}$.

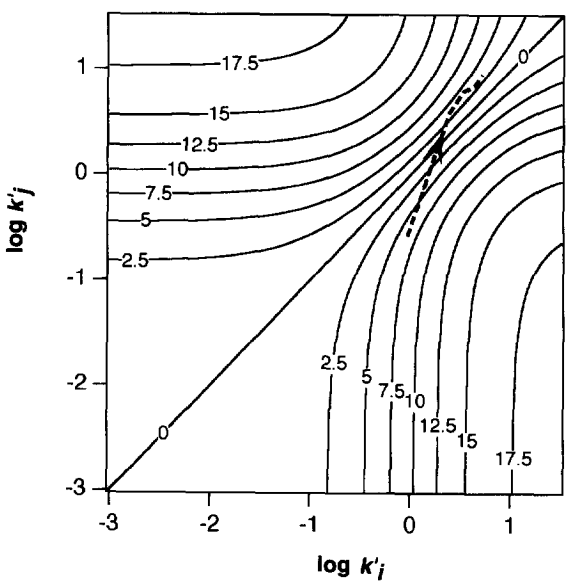

Fig. 2. Iso-resolution plot as a function of k'_i and k'_j (logarithmic scale) for two equally charged (cationic) solutes (Eq. (15)). The value of N is set to 10 000, both t_{m0i} and t_{m0j} are set to 2.34 and t_{mc} is set to 10. See also case 1, Section 2.2.3. The dotted line represents the trajectory of the resolution of the pair 3,4-dihydroxybenzylamine (solute i)–dopamine (solute j). The arrow indicates the direction of increasing concentration of SDS.

This can be explained by a decrease or increase of the separation window (due to the last term on the right-hand side in Eq. (15)).

2.2.4. Case 2: $t_{m0i} < t_{m0j}$

The electrophoretic mobility of a solute is defined by its net charge and the frictional drag, which can be expressed as molecular weight, mass or volume [14–17]. If two solutes differ in charge and/or friction factor, their electrophoretic mobilities are different which can be measured as variations in the migration times in a free zone system. The observed difference is not only determined by these physicochemical properties but also by the experimental settings such as the polarity of the voltage, pH, electroosmotic mobility and the presence of coatings to reduce or inverse the electroosmotic flow. As a result, many different combinations of these parameters can be classified as belonging to case 2 (Section 2.2.4). Calculation of the iso-resolution plot requires values for t_{m0i} and t_{m0j} but is not dependent on these experimental conditions.

The iso-resolution plot, calculated according to Eq. (9) and shown in Fig. 3 is different compared to case 1 (see Section 2.2.3). The high degree of symmetry is clearly reduced, the resolution surface cannot be divided into two identical parts reflected over the diagonal and the region where small changes in k' values result in a significant increase of the resolution is shifted. This shift depends on the values chosen for t_{m0i} and t_{m0j}.

At low capacity factor values, separation is achieved based on the individual differences in charge density hence the separation principle simplifies to a free zone mechanism and Eq. (9) reduces to

$$R_s = \frac{\sqrt{N}}{4} \frac{t_{m0j} - t_{m0i}}{t_{m0j}} \qquad k'_i \approx k'_j \approx k' \ll 1 \qquad (16)$$

This region, denoted A in Fig. 3, can be found at the lower left corner of this figure.

If the solutes interact very strongly with the micellar phase, the migration times of both solutes will be close to t_{mc} so that both k'_i and k'_j are large and almost equal and Eq. (17) shows that the resolution then becomes very low.

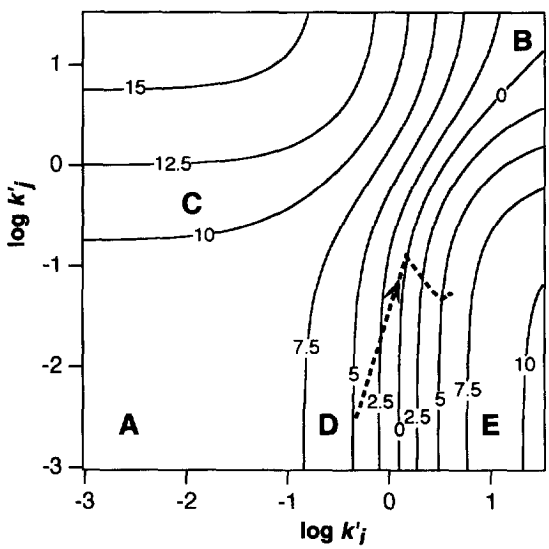

Fig. 3. Iso-resolution plot as a function of k'_i and k'_j (logarithmic scale) for two differently charged solutes in which $t_{m0i} < t_{m0j}$ (Eq. (9)). The value of N is set to 10 000, t_{m0i} and t_{m0j} are set to 3.56 and 5.53, respectively and t_{mc} is set to 10. See also case 2, Section 2.2.4. The dotted line represents the trajectory of the resolution of the pair 2,3-dichlorophenol (solute i)–3-methylbenzoic acid (solute j). The arrow indicates the direction of increasing concentration of SDS.

$$R_s = \frac{\sqrt{N}}{4} \frac{t_{m0j}k'_j - t_{m0i}k'_i + \frac{t_{m0i}t_{m0j}}{t_{mc}}(k'_i - k'_j)}{\frac{t_{m0j}t_{m0i}}{t_{mc}} k'_i k'_j}$$

$$\approx \frac{\sqrt{N}}{4} \frac{t_{m0j} - t_{m0i}}{\frac{t_{m0j}t_{m0i}}{t_{mc}} k'} \approx 0$$

$$k'_i \approx k'_j \approx k' \gg 1 \qquad (17)$$

This region, denoted B in Fig. 3 can be found at the upper right corner of this figure.

At intermediate values of k', the migration velocity of the solute must be seen as a weighted sum of the micellar and the electrophoretic velocity (see also Eq. (4) with X being the weight factor). Consequently, the resolution may either increase or decrease at increasing concentration of the micellar phase and thus capacity factor values, depending on the relative magnitudes of the electrophoretic mobilities and capacity factor values. Therefore, no simplification of Eq. (9) is possible.

If solute j interacts more strongly with the micellar phase than i ($\log k'_j > \log k'_i$), the weight factor X in Eq. (4) is smaller for solute j than for i. From this micellar interaction it follows that solute j will be more retarded in the capillary than i. Since in the free zone system, the migration time of solute j is already larger than i, the difference in the migration times and thus resolution will further increase at higher concentrations of the micellar phase (or increasing k' values). This region of increasing resolutions (compared to a free zone system) is denoted C in Fig. 3.

If solute i interacts more strongly with the micellar phase ($\log k'_j < \log k'_i$), this solute is more retarded by the micelles than solute j. Now the weight factor X in Eq. (4) is larger for j than for i. Solute i, which is the fastest migrating solute in the free zone system, will be, in the presence of micelles, more retarded than j so that the difference in the migration times will decrease at higher concentrations of the micellar phase (or increasing k' values). This region of decreasing resolution (compared to a free zone system) is denoted D in Fig. 3.

It is obvious that if k'_i further increases, the migration time difference will further decrease until both solutes comigrate. This can be seen in Fig. 3 as the line where R_s equals zero. Here, the electrophoretic mobilities and the capacity factors of both solutes are such that the overall migration velocities of both solutes are the same. If the resolution in Eq. (9) is set to zero, the equation can be solved for k'_j which is then expressed as a function of t_{m0i}, t_{m0j} and k'_i

$$k'_j = \frac{t_{m0i} - t_{m0j} + t_{m0i}k'_i\left(1 - \frac{t_{m0j}}{t_{mc}}\right)}{t_{m0j}\left(1 - \frac{t_{m0i}}{t_{mc}}\right)} \qquad (18)$$

This equation can always be solved for any value of t_{m0} and k'_i but only the solutions with a k'_j value higher than or equal to zero are meaningful.

If this difference in k' values increases even more ($\log k'_j \ll \log k'_i$), a peak order reversal occurs and the migration time difference increases again. This region of increasing resolution is denoted E in Fig. 3.

Here the migration mechanism of solute i can be primarily attributed to the micellar interaction, while for solute j both the electrophoretic mobility as well as the micellar interaction contributes to the migration velocity.

In Table 1, a brief overview with a qualitative analysis of the resolution is given for the different situations described above.

The resolution is not necessarily zero if k'_i equals k'_j as in case 1 (Section 2.2.3) and Eq. (9) simplifies to

$$R_s = \frac{\sqrt{N}}{4} \frac{(t_{m0j} - t_{m0i})}{t_{m0j}\left(1 + \frac{t_{m0i}}{t_{mc}} k'\right)} \qquad k'_i = k'_j = k' \quad (19)$$

From this equation it follows that R_s will reach a maximum for $k' = 0$. If the micellar interaction increases equally for both solutes ($k'_i = k'_j$), the differences in electrophoretic properties will become less significant with respect to the overall migration behaviour and therefore the resolution will decrease.

Furthermore, maximisation of the difference between k'_i and k'_j does not necessarily result in an increase in the resolution as is the case for uncharged or equally charged solutes. Again, both electrophoretic behaviour and micellar interaction must be considered to describe the migration.

2.2.5. Case 3: $t_{m0i} > t_{m0j}$

The iso-resolution plot, shown in Fig. 4, is similar to that of case 2 (Section 2.2.4). However, due to the reversal of the relative magnitude of the t_{m0} values, the narrow region of low resolutions is now found at relatively high values of k'_j and relatively low values of k'_i. Although the experimental set-up can be quite different from that in case 2, (Section 2.2.4) both cases can be treated in an analogous way and all the different regions that can be distinguished in Fig. 3 can also be identified in Fig. 4.

In all the situations described above, the resolution becomes worse when both k'_i and k'_j increase to relatively high values. High capacity factors should therefore always be avoided, not only because of an increase in migration time but also because of the loss of separation.

Differently charged solutes can be separated at low capacity factor values, a free zone experiment should then be preferred in stead of a micellar system. However, if the electrophoretic differences are very small or if mixtures of more than two solutes, charged and/or uncharged, must be sepa-

Table 1
Evaluation of the resolution as a function of the relative magnitude of the capacity factors (see case 2, Section 2.2.4)

	Region in Fig. 3	R_s	Remark
$k'_i = k'_j = k' = 0$	A	+	Separation is based on a free zone mechanism and defined by the difference between t_{m0i} and t_{m0j} (see also Eq. 16).
k'_i and $k'_j \gg 0$	B	0	The interaction between the solutes and the micelle is extremely high, hence the overall migration velocity of both solutes equals the migration velocity of the micellar phase.
$k'_j > k'_i$	C	++	Solute j, which is the slowest migrating solute in a free zone experiment, is even slower in a micellar system.
$k'_j < k'_i$	D	-	Migration of solute j is mainly determined by electrophoretic properties and migration of solute i is mainly determined by micellar interaction: these two migration mechanisms partly compensate each other, resulting in a decrease in R_s.
$k'_j = \frac{t_{m0i} - t_{m0j} + t_{m0i} k'_i \left(1 - \frac{t_{m0j}}{t_{mc}}\right)}{t_{m0j}\left(1 - \frac{t_{m0i}}{t_{mc}}\right)}$	/	0	The micellar interaction of solute i results in an increase in the migration time but this is counterbalanced by the electrophoretic properties of solute j. This equation (Eq. 18) can be derived from Eq. 9.
$k'_j \ll k'_i$	E	+	After comigration, a change in the peak order occurs followed by an increase of the resolution.

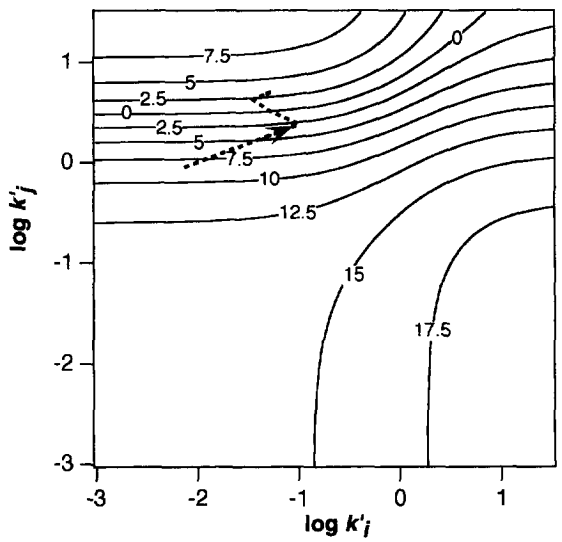

Fig. 4. Iso-resolution plot as a function of k'_i and k'_j (logarithmic scale) for two differently charged solutes in which $t_{m0i} > t_{m0j}$ (Eq. (9)). The value of N is set to 10 000, t_{m0i} and t_{m0j} are set to 5.53 and 2.34 respectively and t_{mc} is set to 10. See also case 3, Section 2.2.5. The dotted line represents the trajectory of the resolution of the pair 3-methylbenzoic acid (solute i)–3,4-dihydroxybenzylamine (solute j). The arrow indicates the direction of increasing concentration of SDS.

rated, the use of a micellar system is a viable alternative.

3. Experimental verification

The approach described in the theoretical part provides general guidelines concerning the (desired) migration behaviour of solutes. However, there are only roughly defined relations between a desired capacity factor and the experimental settings in CE [8,11,18–23] to actually obtain this capacity factor. Nevertheless, the equations describing the micellar interaction and the resolution for charged solutes in MECC are helpful in understanding migration and separation principles and to evaluate changes in various experimental parameters.

To illustrate this, migration data of five solutes at different concentrations of SDS were analysed using the equations presented above. These experiments were performed on a home-built system. An uncoated fused-silica capillary, 58 cm (44.5 cm effective length)×53 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA), thermostated at 40°C was used. All runs were performed in a phosphate buffer at pH 7 and an ionic strength of 0.05 *M*. The samples were injected hydrodynamically, raising the sample vial 5 cm for 20 s. A constant voltage of 18 kV was applied and the runs were monitored at 210 nm.

The observed migration times, together with t_{eo} and t_{mc}, are shown in Fig. 5. 4-Methylcatechol is not charged at the pH chosen and migrates at the speed of the electroosmotic flow in the free zone system. The two cationic solutes, 3,4-dihydroxybenzylamine and dopamine, migrate faster than the electroosmotic flow and are characterised by the same electrophoretic mobility. 2,3-Dichlorophenol and 3-methylbenzoic acid are negatively charged and migrate significantly slower than the electroosmotic flow. Furthermore, the migration time difference at zero SDS concentration reveals that 3-methylbenzoic acid has a higher electrophoretic mobility than 2,3-dichlorophenol.

It can be seen that several changes of the order of migration occur at increasing concentrations of surfactant. For example, changes of the relative peak

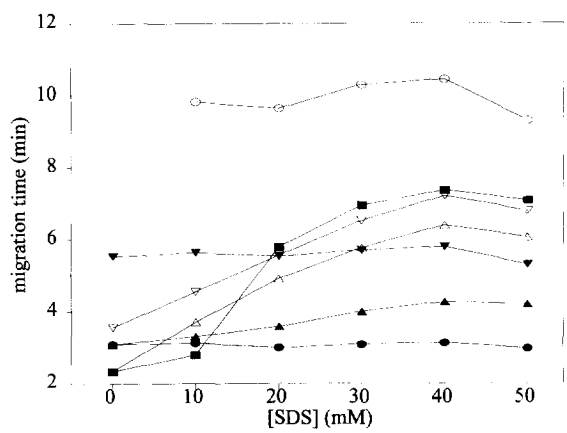

Fig. 5. Migration times of the test solutes, the electroosmotic flow and the pseudo-stationary micellar phase as a function of the concentration of SDS. All experiments were performed in an uncoated fused-silica capillary (ID: 53 μm, total length: 58 cm, length to detector window: 44.5 cm) using a phosphate buffer at pH 7.0 and variable concentrations of SDS. Solutes: 3-methylbenzoic acid (▼), 2,3-dichlorophenol (▽), 4-methylcatechol (▲), 3,4-dihydroxybenzylamine (△), dopamine (■), t_{eo} (●) and t_{mc} (○). These data were kindly provided by J. K. Strasters.

positions are recognised for the peak pairs 2,3-dichlorophenol–3-methylbenzoic acid and 3,4-dihydroxybenzylamine–4-methylcatechol, close to 20 m*M* and 8 m*M* SDS, respectively. Even more dramatic is the migration behaviour of dopamine, which is the fastest migrating solute in the free zone system, but the slowest migrating solute at high concentrations of SDS.

From these migration data, capacity factors for all solutes can be calculated as shown in Fig. 6. Corrections for the small changes in the electroosmotic flow at higher SDS concentrations are introduced as described in the theoretical section. To calculate the capacity factor for the charged solutes (both anionic and cationic), Eq. (6) should be used.

From the migration times in the free zone system, it follows that 3-methylbenzoic acid has a high negative charge density. Due to electrostatic repulsion with the SDS micelles, there is hardly any interaction between this solute and the surfactant aggregate so that the capacity factor is close to zero. The migration behaviour of 3-methylbenzoic acid is solely determined by the electrophoretic properties of this solute (apart from changes in the electroosmotic flow). Consequently, the migration time of this solute is fairly constant.

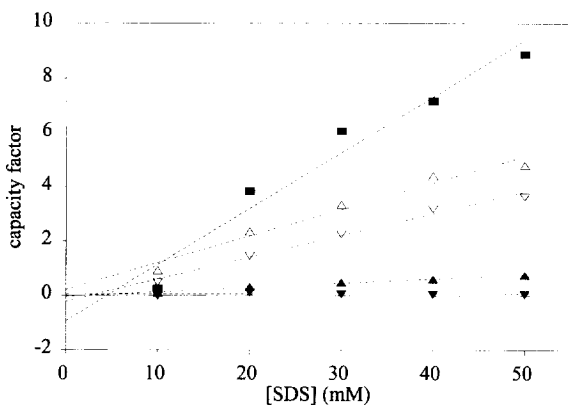

Fig. 6. Capacity factors of the test solutes as a function of the concentration of SDS. The capacity factor values are calculated according to the equation for charged solutes (Eq. (6)). Dotted lines represent the least square fits for the k' values. All experiments were performed in an uncoated fused-silica capillary (ID: 53 μm, total length: 58 cm, length to detector window: 44.5 cm) using a phosphate buffer at pH 7.0 and variable concentrations of SDS. Solutes: 3-methylbenzoic acid (▼), 2,3-dichlorophenol (▽), 4-methylcatechol (▲), 3,4-dihydroxybenzylamine (△) and dopamine (■). These data were kindly provided by J. K. Strasters.

4-Methylcatechol, which is uncharged at pH 7.0, is retarded at higher concentrations of SDS and this can be attributed to the interaction with the micellar phase. Either Eq. (6) for charged solutes or Eq. (1) for uncharged solutes can be used to calculate the value of the capacity factor.

3,4-Dihydroxybenzylamine, dopamine and 2,3-dichlorophenol carry significant charges but show interaction with the micelles as well. Therefore, both the electrophoretic mobility of the solutes as well as the interaction with the micellar phase must be considered to evaluate the migration mechanism of these solutes. The capacity factors of 3,4-dihydroxybenzylamine and dopamine are larger compared to the other solutes. This is probably due to the electrostatic attraction between the positively charged solutes and the oppositely charged micelle.

The linear relationship between the capacity factors and the surfactant concentration is satisfactory (r^2 is typically higher than 0.98) except for 3-methylbenzoic acid but this may be related to the calculation of small capacity factors. The concentration of surfactant at a k' value of zero, results from extrapolation of the regression lines in Fig. 6 and equals the CMC of SDS. Here, typical values around 4 m*M* are found which are smaller than the theoretical value of 8 m*M*. However, it is known that the actual CMC is influenced by buffer properties like the ionic strength and the temperature. The migration data of 3,4-dihydroxybenzylamine do not seem to be suitable for the determination of the CMC (CMC = ± −2.5 m*M*) but the exact reason is not clear.

The resolution according to Eq. (9) was calculated for 3,4-dihydroxybenzylamine–dopamine. The migration properties of this pair of solutes is discussed in the theoretical part under case 1 (Section 2.2.3). The calculated resolution is shown in Fig. 2 as the dotted trajectory. Since both solutes carry the same charge, they cannot be separated in a free zone system. The adequate separation at 10 m*M* SDS is mainly caused by the interaction of 3,4-dihydroxybenzylamine with the micelles. The trajectory in Fig. 2 reveals that the quality of the separation decreases when the surfactant concentration increases above 10 m*M*. This loss of resolution results from a dramatic

increase of the micellar interaction of dopamine. Close to 15 m*M* SDS the solutes comigrate, as they do in the free zone system. Apparently, both solutes are slowed down to the same extent by the micellar interaction resulting in equal capacity factors. Close to 15 m*M* SDS, a peak crossing occurs and 3,4-dihydroxybenzylamine now migrates faster than dopamine. Increasing the concentration of SDS from 20 to 50 m*M* does not result in an improvement of the resolution. As mentioned earlier, high capacity factor values should not be preferred because excessive micellar interaction does not improve the quality of the separation and, in addition, the overall migration times increase. It is obvious that in this case, the separation is solely based on the differences in the micellar interaction. Consequently, a larger difference in the respective k' values results in an increase of the resolution which also results from Fig. 2.

The separation of 2,3-dichlorophenol and 3-methylbenzoic acid is an example of case 2 (Section 2.2.4), as discussed in the theoretical section. Again, the trajectory is shown as a dotted line in Fig. 3. Both solutes are negatively charged but have a different electrophoretic mobility. As mentioned earlier, 3-methylbenzoic acid shows no interaction with the SDS micelles hence k' of 3-methylbenzoic acid is close to zero (log k' between -2.5 and -0.9). The separation at low SDS concentrations is solely due to the differences in the electrophoretic properties. At increasing concentrations of SDS, the migration time difference and thus resolution decreases which can be explained by an increase of the interaction between 2,3-dichlorophenol and the micelles (see also Figs. 5 and 6). Finally, the two peaks overlap at 20 m*M* SDS, as is depicted both in Figs. 3 and 5. In contrast, the capacity factor values, shown in Fig. 6, are continuously diverging as a function of the concentration of SDS hence a comigration of 2,3-dichlorophenol and 3-methylbenzoic acid is not supported by the data. This illustrates that an increase of the resolution for charged solutes cannot always be obtained increasing the difference in capacity factor values as is the case for uncharged or equally charged solutes. At higher concentrations of surfactant, the capacity factor of 2,3-dichlorophenol further increases, resulting in a peak crossing and an increase of the resolution at the expense of the analysis time. This is also revealed in Fig. 3 where the dotted line descends to lower resolution values at low capacity factor values, crosses the valley ($R_s = 0$) and then ascends at the other side towards increasing resolutions at high capacity factor values.

The dotted line in Fig. 4 refers to the separation of two oppositely charged solutes: 3-methylbenzoic acid and 3,4-dihydroxybenzylamine. Because of the large differences in the charge density of both solutes, the separation is quite good in the free zone system and at low SDS concentrations. Increasing the SDS concentration up to 30 m*M* reduces the resolution resulting in a comigration of both solutes. Again, this can be explained by a dramatic increase of the micellar interaction of 3,4-dihydroxybenzylamine. At even higher concentrations of SDS, the peak order reverses and the resolution increases. It is obvious that for an adequate separation of these two solutes, the addition of SDS is not required.

The examples discussed here clearly illustrate that by using the appropriate capacity factor and resolution equations, it is possible to gain more insight in the migration behaviour of charged and neutral solutes in MECC, in particular related to their interactions with the micellar phase. Evidently, the migration behaviour of every (relevant) pair of solutes can be evaluated in this way. Careful examination of the data enables the adjustment of the appropriate parameters, in this case the concentration of SDS, to optimise the separation between various peak pairs. Obviously, for optimisation purposes, smaller sets of experiments should be used to predict the resolution of these peak pairs. Extension to other parameters, like the pH of the buffer, should also be possible.

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