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Review

Optimisation of selectivity in capillary electrophoresis with emphasis on micellar electrokinetic capillary chromatography

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Abstract

Separations in capillary electrophoresis and especially in micellar electrokinetic capillary chromatography are characterised by a large number of parameters and therefore difficult to optimise. This paper reviews recent approaches suitable for optimisation of selectivity in capillary electrophoresis.

Typical features of optimisation strategies applicable to capillary electrophoresis and micellar electrokinetic capillary chromatography in particular are discussed. A distinction is made between statistical approaches, using fitting procedures of polynomial equations, and practical optimisation schemes, based on physicochemical models describing the migration behaviour.

Besides speeding up the search in finding satisfactory separation conditions, additional knowledge may be obtained about the migration and separation mechanism(s) when a systematic approach is applied. However, due to the complexity and the number of available optimisation schemes, these approaches should not be used as black-box systems. The analyst has a crucial role in optimising a separation.

Contents

1. Introduction

Capillary electrophoresis (CE) is a rapidly expanding analytical technique that can be used to separate many different compounds. The separation of biological molecules such as peptides, proteins [1–6] and nucleic acids [7,8] as well as inorganic ions [9,10] and pollutants [11,12] has been reported in the literature. In spite of the fact that CE is characterised by a high efficiency, the desired separation is often only obtained after considerable experimentation. Although the adjustment of system parameters, like sample characteristics to introduce

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stacking [13,14], the use of electrokinetic injection with its discriminating capabilities [15,16] or the selection of an appropriate detection wavelength for detection or spectral recognition [17,18], should not be overlooked, system optimisation will not be dealt with. The purpose of the present review is to give an overview of the guidelines and strategies that are currently available to achieve an adequate selectivity with the minimum number of experiments.

Selectivity in capillary zone electrophoresis (CZE) is strongly influenced by the pH of the buffer. In addition, the type and concentration of buffer and the presence of an organic modifier can affect both selectivity and efficiency. In micellar electrokinetic capillary chromatography (MECC), a special mode of CE [19], two additional parameters have a remarkable influence on the separation, i.e. the type and concentration of surfactant. As a result numerous factors will affect MECC experiments.

The temperature affects different physicochemical parameters like viscosity, pK_a and pH values, absolute mobilities and the critical micellar concentration (cmc) of various surfactants and thus the separation. As a result, an efficient temperature control is inevitable in method development and although temperature changes can be used to improve the selectivity [20,21], we will focus on methods in which the temperature is assumed to be constant.

Clearly, an appropriate optimisation strategy should be used to find good separation conditions in the shortest time and/or after only a few experiments. Basically, all optimisation strategies consist of three distinct steps: the choice of the appropriate parameter(s) and the parameter space, a model or algorithm to describe the migration behaviour and a criterion to evaluate the resulting electropherogram. For an overview the reader is referred to Ref. [22].

The choice of the parameter(s) to be optimised is mainly influenced by the analytical technique itself and therefore, this choice often seems quite obvious. In contrast, the choice of the limits of each parameter, which usually defines the parameter space, is more difficult to rationalise. Although generally the absolute minimum and maximum value of a parameter is physically defined, these limits seldom will be the actual limits used in the optimisation procedure. For example, optimisation of the pH as the parameter having much influence on selectivity in CZE is a rather obvious choice. However, the actual pH range under investigation is difficult to define without relevant knowledge about the sample to separate. In this respect dissociation constants as well as pH stability data are essential in CZE. Unfortunately, in many cases relevant data concerning the sample under investigation are not available so that the experience of the analyst becomes important.

After the choice of the appropriate parameters and their limiting values, experiments must be performed to explore the migration of the solutes as a function of the parameter(s). A description of the behaviour of the analytical system in the entire parameter space is then generally obtained by interpolation using an algorithm that relates the migration of the solutes to the parameter(s). Differences can be found among the various models used and the theoretical basis underlying the model can be quite different. Approaches based on physicochemical properties like dissociation constants, mobility data and diffusion coefficients of the solutes and buffer properties like ionic strength and pH as well as models based on strictly mathematical equations, treated in a statistical way, are reported. It is clear that there is a correlation between the accuracy of the model and the time and effort it takes to satisfactorily predict the migration behaviour of different solutes and hence to find good separation conditions.

The final step in the optimisation strategy is the evaluation of the migration behaviour of the solutes predicted in the parameter space in terms of the quality of the separation. The goal of an optimisation may vary considerably from one case to another, e.g. the separation of two enantiomers requires a different criterion than a peptide map in which many different unknown solutes must be detected. Therefore, this goal must be translated into appropriate objective mathematical functions, defining the criterion. The criterion relates the quality of an observed

electropherogram to a desired one and this choice is critical and affects both the optimisation procedure to follow and the results obtained. Furthermore, it is not necessary to search for the global optimum in the parameter space but it satisfies to find experimental conditions resulting in sufficient separation. Many different criteria are proposed in the literature and some of them, which are used in the optimisation of chromatographic experiments but are also useful in CE, can be found in Ref. [22]. Recently, Hayashi et al. [23] studied the precision and throughput in MECC and concluded that these statistical parameters are suitable as criteria in MECC. The criterion should always be carefully evaluated in relation to the ultimate goal of the analyst.

Although for high-performance liquid chromatography (HPLC) several optimisation schemes have been published [22], these approaches often require substantial modification before they can be used to optimise a CE separation. Recently, some books on capillary electrophoresis have paid some attention to method development and optimisation strategies in CE [24–26]. In the first part of this review the possibility to apply statistical approaches is discussed, while in the second part some feasible optimisation schemes are commented.

2. Statistical approaches in the optimisation of CE

The optimisation of CE and especially MECC experiments is complex due to the number of parameters affecting the separation. Further complications can arise from the mutual interaction of the parameters. Examples illustrating this phenomenon have been reported both for micellar liquid chromatography [27] and MECC [24]. This explains why the development of physicochemical models describing the separation mechanisms is not an easy task. Often the behaviour of the system is approximated by simple mathematical equations for which only a minimum amount of knowledge is required.

An example of such an approach is the use of a simplex algorithm. The principle of a simplex method is covered extensively by the literature [22,28]. In general, the simultaneous optimisation of n parameters results from a fitting procedure of the response (or criterion) y with a first-order model to the parameters x as shown in Eq. 1.

$$y = b_0 + b_1x_1 + b_2x_2 + \cdots + b_n\mathrm{x}_n \quad (1)$$

The optimum is approached in a sequential way constructing geometrical figures (called simplex) in the parameter space using previous experimental results. These sequences are repeated until the separation is satisfactory or until no further improvement is observed.

The advantage of this approach is that it is applicable to any type and number of parameters and that knowledge about the separation mechanism is not required to calculate the response or define the parameter settings for the next measurement. However, an important drawback of the simplex method is the large number of experiments that is generally needed to reach the optimum. This is clearly illustrated by Castagnola et al. [29], who optimised pH, concentration of organic modifier and concentration of surfactant in the separation of derivatised amino acids. Although the variable-sized weighted simplex optimisation design was used to speed up the procedure, still 10 to 15 steps were required to reach separation conditions that are satisfactory in terms of the mean resolution of all the relevant peak pairs. In addition, the choice of the starting conditions is very critical since different starting conditions can lead to different solutions. Finally, it should be noted that by applying simplex methods much information is lost since only the information of the last $n + 1$ experiments is retained. An adequate description of the response surface is not obtained in this way and this is a serious disadvantage when the response surface is complex.

Another multi-parameter optimisation procedure, called the overlapping resolution mapping scheme (ORM), was introduced for CE by Li and co-workers [30–35]. After defining the parameters and the accompanying parameter

space, the initial experiments are performed, and the response (resolution R_s) of each peak pair is used to determine the coefficients of a polynomial equation that not only accounts for the effect of each parameter but also includes mathematical interaction effects between the parameters, expressed as $x_i x_j$-terms in Eq. 2:

$$R_s = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \quad (2)$$

Once the coefficients of the polynomial equations are known, the resolution for each peak pair over the whole parameter space can be predicted and visualised as a resolution map. The optimum separation conditions can then be deduced from an overlay of all the resolution maps. Applications of this method include the separation of solutes such as sulphonamides [30,31], flavonoids [31,32], derivatised amino acids [33,34], drug substances [34] and porphyrins [35].

It should be realised that in this strategy peaks are not identified, hence the actual migration behaviour of the solutes is not followed. Significant errors may result from changes in the relative peak positions. This problem was discerned by Glajch et al. [36], who initially developed the ORM approach for HPLC. Due to such effects the response surface may be complex and discontinuous, and overlapping of the resolution maps can then be expected to yield unreliable results. For this reason peak tracking should be considered as a valuable asset, and this task is much facilitated by using advanced detection techniques such as diode-array spectrophotometry or mass spectrometry.

A third type of experimental design that has been shown to be useful for optimisation purposes is the Placket–Burman statistical design, which is a fractional factorial design that can be used if the number of parameters is one less than a multiple of four. Dummies should be added to meet the required number of parameters. A dummy can be used to estimate the variability of the system and the significance of the effects found for the true physical parameters. Statistical treatment of the data can often be used for the screening of many parameters and the models used to describe the results of the experiments are typically first-order in each parameter. The most important parameters found with this screening procedure can then be studied in a full multi-level factorial design.

Vindevogel and Sandra [37] used this approach to obtain a satisfactory separation of a mixture of testosterone esters. Seven parameters are evaluated by means of eight initial experiments in which the effect of pH and the concentrations of buffer, acetonitrile, sodium dodecyl sulphate (SDS) and sodium heptyl sulphate on the analysis time, the noise, the efficiency and the resolution are studied. Interpretation of the results should, however, be done very carefully since the observed changes in migration behaviour may be due to multiple interactions.

An important advantage of these factorial-design type of procedures is that they are applicable under many different experimental circumstances and that there is no restriction concerning the type of solutes and parameters in the optimisation. However, since in this way no general rules are obtained concerning migration mechanisms, the results are restricted to the separation under investigation. A change in the separation conditions requires that the whole procedure has to be followed over again.

3. Optimisation procedures based on physicochemical models

When relevant knowledge of the mechanism of a given type of separation is available, optimisation protocols can be developed that make use of these separation principles, expressed by an appropriate algorithm.

In Section 3.1, approaches in which fundamental equations describing the migration behaviour and the resolution will be treated. These general equations are based on a theoretical description of the separation process. The parameters describing the migration are then evaluated and adjusted to reach a maximum value of the resolution. In such a way, global guidelines,

pointing to the desired migration behaviour, can be formulated. However, the translation of these guidelines into practical separation conditions is often not obvious, and therefore the practical applicability is limited.

In Section 3.2, specific physicochemical models are shown describing the migration behaviour of particular solutes as a function of one or more parameters. The experimental separation conditions can then be adjusted in such a way that the criterion reaches satisfying values. Clearly, these procedures are suitable to solve practical optimisation problems.

3.1. Global approaches in the optimisation of CE

In MECC uncharged solutes are separated according to differences in micellar solubility. In analogy with HPLC, the equations describing the capacity factor k' and the resolution R_s in MECC are based on a classical chromatographic description as shown in Eqs. 3 and 4 [38].

$$k' = \frac{t_r - t_{eo}}{t_{eo}(1 - t_r/t_{mc})} \tag{3}$$

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_j}{1 + k'_j} \cdot \frac{1 - t_{eo}/t_{mc}}{1 + (t_{eo}/t_{mc})k'_i} \tag{4}$$

Here, t_r is the migration time of the solute, t_{mc} is the migration time of a solute totally solubilised in the micelles (e.g. Sudan III), t_{eo} is the migration time of an uncharged solute that has no interaction at all with the micelles (e.g. methanol), α is the selectivity and defined as the ratio of two capacity factors, N is the number of theoretical plates and subscripts i and j denote two (closely migrating) compounds. The definition of the capacity factor k' is clearly analogous to the conventional chromatographic definition of the capacity factor as expressed in Eq. 5:

$$k'_{chrom.} = \frac{t_r - t_0}{t_0} \tag{5}$$

where t_0 is the retention time of a non-retained solute and the additional term in the denominator in Eq. 3 accounts for the size of the migration window in MECC.

It is obvious that this limited migration range, expressed as the ratio t_{mc} over t_{eo}, is important with respect to the peak capacity and separation capabilities of a micellar system. This is illustrated in Fig. 1, showing three simulated electropherograms of two solutes having identical α-values but different values of k'. The separation is superior at intermediate values of k' (electropherogram B in Fig. 1). Low capacity factors result in relatively small micellar interactions and a lack of selectivity and high capacity factor

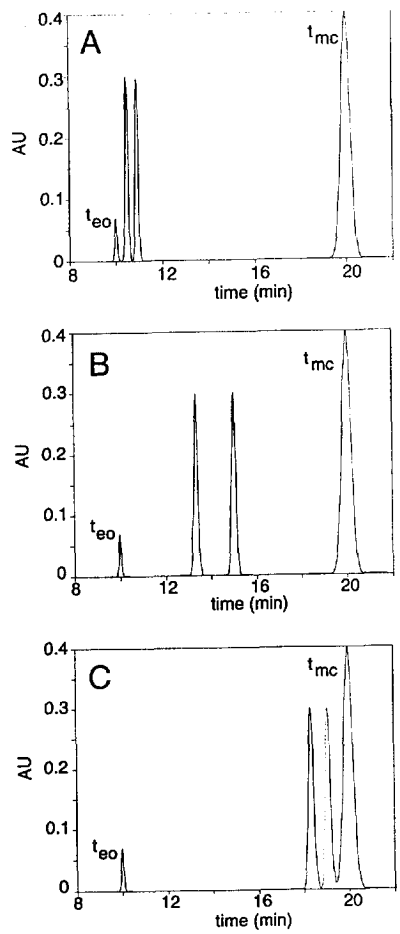

Fig. 1. Simulated electropherograms illustrating the drawbacks of a limited elution range, typical for MECC. Both the migration window ($t_{mc}/t_{eo} = 2$) and the selectivity ($\alpha = 2$) are held constant. (A) $k'_i = 0.1$; $k'_j = 0.2$. (B) $k'_i = 1$; $k'_j = 2$. (C) $k'_i = 10$; $k'_j = 20$.

values result in longer migration times as well as in bad separations because all the solutes are migrating close to t_{mc}.

Extension of the migration window can be achieved by altering the electroosmotic and/or the micellar electrophoretic mobility [39,40]. Recently, Ahuja et al. [41] demonstrated the use of a mixed pseudo-stationary micellar phase of SDS and Brij 35. The electroosmotic and micellar electrophoretic mobilities are matched by adjusting the ratio of the concentration of Brij 35 and SDS so that the micellar mobility equals the electroosmotic mobility but has the opposite sign. This results in a real stationary micellar phase and an infinite migration range is obtained, even at relatively high pH values (ca. 7) where electroosmotic velocities are significant.

The prediction of conditions for optimal separation of neutrals in MECC, formulated by Foley [42] is based on the assumption that selectivity is mainly determined by the partitioning of the neutral solutes between the water and the micellar phase. Accordingly, the concentration of surfactant is the most important parameter to be optimised in that case.

The surfactant concentration $[M]$ is related to the capacity factor k' as shown in Eq. 6:

$$[M] = \frac{k' + v \cdot cmc \cdot (k' + P_{wm})}{v \cdot (k' + P_{wm})} \approx \frac{k'}{P_{wm} \cdot v} + cmc, \quad P_{wm} > k' \tag{6}$$

where P_{wm} is the partition coefficient of a given solute for the water and the micellar phase, v is the partial molar volume of the surfactant and cmc is the critical micelle concentration. Assuming that both N and α are independent of k', the optimum capacity factor $k'_{opt(R_s)}$ is derived from the classical resolution equation (Eq. 4):

$$k'_{opt(R_s)} = \sqrt{\frac{t_{mc}}{t_{eo}}} \tag{7}$$

Substitution of $k'_{opt(R_s)}$ in Eqs. 6 and 4 allows the calculation of the optimal surfactant concentration (equation not shown) and the corresponding best resolution, respectively:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{\sqrt{t_{mc}/t_{eo}} - \sqrt{t_{eo}/t_{mc}}}{2 + \sqrt{t_{mc}/t_{eo}} + \sqrt{t_{eo}/t_{mc}}} \tag{8}$$

By adjusting the concentration of surfactant it is possible to optimise a separation, independent of the hydrophobicity of the solutes. The same can be done to optimise the resolution per unit time (equations not shown). It is obvious that both approaches do not predict the same optimal parameter settings.

The authors conclude that there is an optimal region for the capacity factor. In general, intermediate values of k' are preferable. Low values of k' will lead to a short analysis time but will suffer from bad resolution, except for samples that are very easy to separate. High capacity factors result in excessive retention and a loss in resolution since all the solutes migrate close to t_{mc}. This phenomenon is not known in conventional column chromatography.

Ghowsi et al. [43] have rewritten the equations based on chromatographic principles using an electrophoretic approach in MECC for neutral solutes. The resolution is not only expressed as a function of the capacity factor but also as a function of μ^*_{ep}, which is the average effective electrophoretic mobility of a neutral solute. N is dependent on the capacity factor and thus on the effective migration time of each solute separately, and in addition, the efficiency is characterised by a Van Deemter-like behaviour of plate number versus voltage.

Using these equations Ghowsi et al. [43] performed a theoretical optimisation for three modes of MECC differing in the net migration velocity of the micelles. Although the approach and the accompanying equations are quite different compared to the optimisation procedure of Foley [42], the results are very similar: when the micelles move to the positive electrode, resolution is maximal if the neutral solute is carried by the micelles to the same extent as the solute is carried by the electroosmotic flow in the opposite direction. Obviously, the analysis time will then be infinite. When the micelles are stationary or moving to the negative electrode, the optimum capacity factor can be calculated by deriving the appropriate equations, and it can be

shown that maximum resolution will be reached for k' values close to 5. In a more qualitative way Terabe et al. [38] came to a similar conclusion starting with equations derived for conventional chromatography.

Based on these global guidelines, together with many experimental observations, Terabe [44] has formulated an introductory guide for optimisation in MECC, which is summarised by the flow chart in Fig. 2. Here, theoretical knowledge is translated in experimental CE-conditions. The first experiment is performed with standard MECC-conditions, and based on this result, the analyst is advised to change the experimental settings concerning the type and concentration of surfactant or other buffer additives so that optimal capacity factor values are approached. The applicability is demonstrated by Bevan et al. [45], who optimised the resolution for mixtures of synthetic oligonucleotides. Although small deviations from theory were observed, high concentrations of urea at various SDS-concentrations, organic modifiers and the use of bile salts and cationic micelles could be used to control the capacity factors and increase the chances to find good separation conditions.

3.2. Practical approaches in the optimisation of CE

It is obvious that for charged solutes the pH will be the first parameter of choice. Kenndler and Friedl [46] have derived a relation between the resolution of monovalent ions in CZE and the pH of the buffer. Both selectivity and efficiency depend on the charge number, and thus for weak electrolytes on the pH as expressed by Eq. 9 for two solutes i and j:

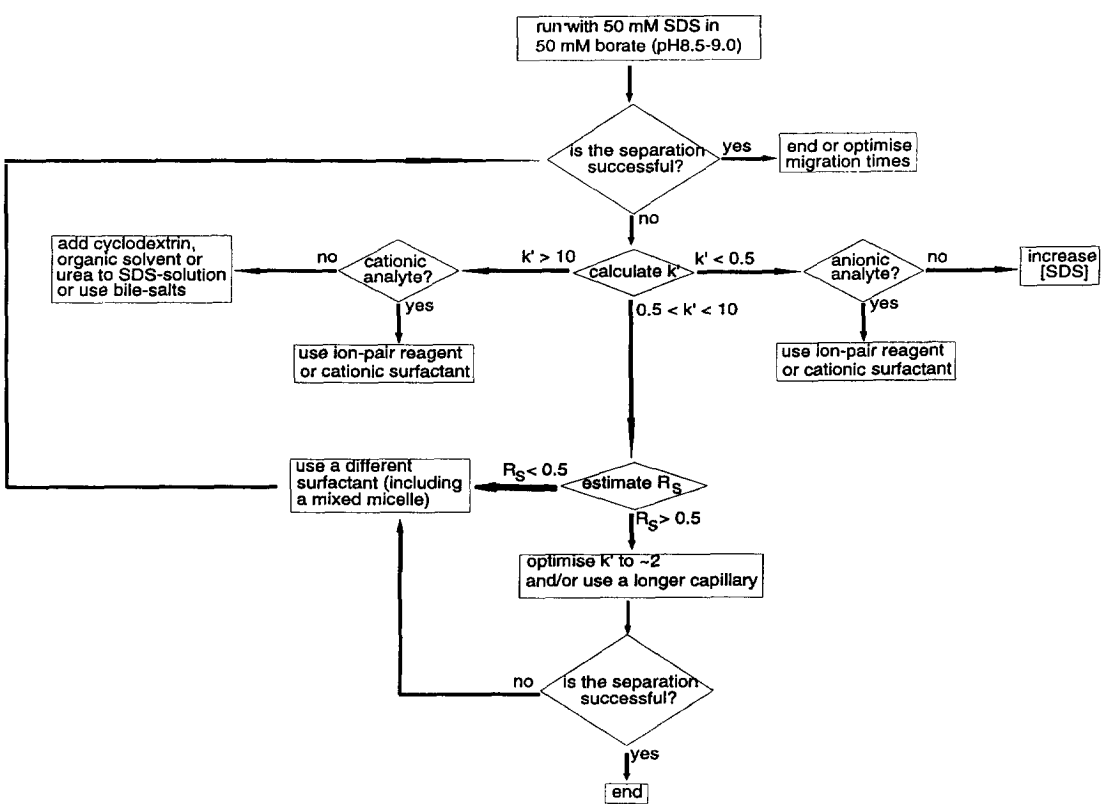

Fig. 2. Introductory guide to the method development of a MECC experiment formulated by Terabe [44].

$$R_{ji} = \frac{\left(\frac{\mu_{act,i}}{\mu_{act,j}} - 1\right) + \left(\frac{\mu_{act,i}}{\mu_{act,j}}\Delta_j - \Delta_i\right)}{(1-\Delta_i)^{3/2} + \frac{\mu_{act,i}}{\mu_{act,j}}(1-\Delta_j)^{3/2}} \sqrt{\frac{e_0 U}{32kT}} \quad (9)$$

where μ_{act} is the actual mobility, $\Delta_i = 10^{pK_{a,i}-pH}$, $\Delta_j = 10^{pK_{a,j}-pH}$, e_0 is the electric charge, k is the Boltzmann constant, T is the absolute temperature and U is the applied voltage. This equation was also extended to multivalent ions [47]. The resolution as a function of pH of the buffer and applied voltage was calculated for different solutes and optimal settings can easily be deduced. As expected, the predicted resolution is a complex function of the pH in the case of multivalent ions.

Use of these relations requires accurate knowledge about the acid–base properties of the solutes and in the study cited above, the relevant pK_a values were taken from the literature. In addition, the mobilities of the solutes have to be known very precisely, and since not much literature data is available, these values should be determined experimentally by measuring the migration time of a solute as a function of the pH. Obviously, this requires a large number of experiments.

It is recognised by the authors that the model proposed is limited by the lack of consistent data on the analyte properties [48]. Nevertheless, both for mono- and multivalent ions an optimal pH was predicted, resulting in a baseline separation of the entire series of substituted benzoic acids and phenols investigated. It was found that a change of the pH as small as 0.04 may have a dramatic influence on the resolution, and in spite of uncertainties in both the mobilities and the dissociation constants, this effect is predicted by this approach.

Jacquier et al. [49] also optimise the pH for the separation of monovalent ions. With only one experiment when the pK_a value is known and two experiments when the dissociation constant is unknown, the migration time of a solute over the pH range can be predicted. In addition, an estimate of the molecular diffusion coefficient and hence the peak width is obtained. The electroosmotic flow is modelled in a very simple way using the dissociation constant of the silanol groups.

Although in this approach the number of experiments is very limited and also some uncertainty remains concerning the electroosmotic mobility and the dissociation constants, the predicted behaviour of the solutes is in reasonable agreement with the experimental results. Using this strategy, the separation of three different solute mixtures: three chlorophenol geometric isomers, three nitrophenol geometric isomers and three chloroaniline geometric isomers was rapidly achieved.

The mobility of several monovalent substituted phenols is predicted by Smith and Khaledi [50] modelling the electrophoretic mobility μ_{ep} as a function of the pH of the buffer and the acid dissociation constant K_a:

$$\mu_{ep} = \mu_{A^-} \cdot \frac{K_a/[H^+]}{1 + K_a/[H^+]} \quad (10)$$

where μ_{A^-} is the electrophoretic mobility of the anionic form of the acid. The parameters μ_{A^-} and K_a can be determined by fitting Eq. 10 to the measured μ_{ep} as a function of pH.

Although this approach is analogous to that of Friedl and Kenndler [47], there are also some important differences. The pK_a values obtained by fitting Eq. 10 to the experimental data are apparent dissociation constants depending on the actual CE conditions, and they are not necessarily close to literature data. (Titration data illustrate that for amino acids and small peptides apparent dissociation constants may differ significantly [51], illustrating the effect of the surfactant on the dissociation behaviour of these solutes.) As few as four measurements may be sufficient to obtain a reliable fit. Here, the choice of the pH range to be scanned is important and is more easy when physicochemical data of the solutes are available. Limitations on the actual prediction of migration times is discerned so that the migration order prediction for closely migrating peaks may fail, especially for solutes having almost identical pK_a or mobility values.

The well-known observed linear relation between the capacity factor and the concentration of surfactant in MECC was used by Pyell and

Bütehorn [52] to increase resolution. Two experiments at different SDS concentrations enable the calculation of k' and thus migration times of all the solutes at different concentrations of SDS. This one-parameter optimisation procedure resulted in baseline separation of a mixture of seven methylnitroanilines. Furthermore, the optimisation of the concentration of modifiers like urea and glucose was performed in a similar way, using appropriate logarithmic relations between the migration and the concentration of the modifier.

The description of the behaviour of ionisable solutes in a micellar system is complicated due to the combination of the electrophoretic and chromatographic migration mechanisms. Khaledi and co-workers extended the procedure described earlier to the separation of both negatively [53–55] and positively [55,56] charged solutes in a micellar system. Here, the two important parameters are pH and the micellar concentration. Assuming that the net migration of an ionisable solute is the weighted average of the migration parameter of the solute in the associated (a) or non-associated (b) forms both in the aqueous and micellar phase, the net mobility of an ionisable solute can be expressed as:

$$\mu_{ep} = F_{aq,a} \cdot \mu_{aq,a} + F_{aq,b} \cdot \mu_{aq,b} + (F_{mc,a} + F_{mc,b}) \cdot \mu_{mc} \quad (11)$$

where μ_{aq} is the mobility of the solute in the aqueous phase in the associated and non-associated forms, respectively (subscripts a and b), μ_{mc} is the mobility of the micelle and the F-values are the mole fractions of the solute in the micellar and aqueous phase (subscripts mc and aq) in the associated and non-associated forms. Ion-pair formation between the charged solute and the oppositely charged surfactant constitutes an additional mechanism affecting migration, which is also considered. Note that this ion-pair complex and the uncharged solute molecules in the aqueous phase are assumed to migrate with the electroosmotic velocity and that μ_{eo} is not included in Eq. 11 since it is not important in the estimation of the electrophoretic mobility. Rewriting Eq. 11 results in a general expression which relates the mobility of a solute to all

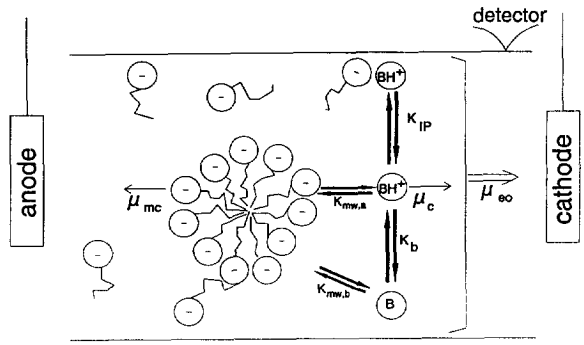

Fig. 3. Relevant equilibria of a cationic solute (BH^+ and B) in a micellar system containing negatively charged micelles. From Ref. [55].

possible equilibrium constants, acid–base dissociation constants, pH and mobilities of the micellar phase and the solute under investigation (equation not shown). Four situations can be distinguished: an acid or basic solute that migrates in a micellar system containing positively or negatively charged micelles. For each case, the general mobility expression is then rewritten and simplified, e.g. ion-pair formation is only considered for oppositely charged solute and surfactant molecules.

As an example, the relevant interactions between a weak base and a negatively charged surfactant are schematically illustrated in Fig. 3. Consequently, the relevant apparent parameters are estimated using five experiments at different pH and SDS concentration settings. Subsequently, the mobility of each solute can be predicted over the entire pH and SDS range. The equations were experimentally verified and the results are briefly summarised in Table 1. Note that fine-tuning of the separation of the aromatic amines was achieved by adding SDS and acetonitrile and this is done independent of the proposed optimisation strategy, and this means that insight in the separation mechanisms is required.

4. Conclusions

The use of a systematic optimisation strategy in the development of CE applications is highly recommended and should be preferred to trial and error. In this way satisfactory separation

Table 1
Predicting capabilities of migration times of charged solutes in MECC using the phenomenological approach developed by the group of Khaledi

Solute–buffer system	Predicting capabilities	Remark
Acidic solutes–anionic micelle	++	–
Basic solutes–anionic micelle	+	Ion-pair formation is assumed to be very important and free BH^+ is not present The separation is further improved increasing the SDS concentration (improved peak shape) and adding 10% acetonitrile (extends the migration window)

conditions are established in the shortest time and/or with only a few experiments. Furthermore, detailed information on the separation mechanisms may be obtained. This will be of great use in the development of different applications by the analyst and will facilitate the improvement of existing optimisation schemes and the introduction of new and better strategies in method development.

At present there is a wide choice of optimisation procedures. For some of these strategies only a minimal knowledge of separation principles is needed, while other approaches, based on the assumption of a particular separation mechanism, require more specific information about the solutes to separate. The choice of a particular strategy depends on the goal of the analyst and the feasibility of such an approach.

An important aspect of optimisation is the proper choice of the parameter(s) and the parameter space. In some cases the significance of this aspect seems to be underestimated, but, unfortunately, general rules do not exist. The choice of the type of parameter may seem obvious in most cases but the choice of the parameter space is more complicated, more difficult to justify and often influenced by the analyst's experience. It should be realised that badly chosen "start conditions" may drastically reduce the chances to find useful separation conditions. Due to the high complexity and the linking of the parameters, a multi-parameter approach in which several parameters are optimised simultaneously in combination with a peak identification procedure should be favoured.

More knowledge of CE separations makes predictions of the migration behaviour more accurate, but a good balance should be found between the required knowledge of the analytical system and the effort required to obtain it. In some cases this knowledge is already included in the optimisation approach by the designer, so it does not need to be provided by the analyst. However, the lack of physicochemical data often hampers such optimisation protocols.

None of the strategies discussed here can be used as a black-box or a stand-alone system. The analyst himself is an important factor, and by choosing the appropriate optimisation strategy he has a major influence on the outcome of the optimisation procedure. Adequate interpretation of the measurements can be of decisive importance. Therefore it remains crucial that the strategy or the algorithm as well as the separation technique is known in detail by the analyst.

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