ELSEVIER

Journal of Chromatography A, 867 (2000) 247–259

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Resolution optimisation in micellar electrokinetic chromatography using empirical models

P.V. van Zomeren, M.J. Hilhorst, P.M.J. Coenegracht, G.J. de Jong*

*Department of Analytical Chemistry and Toxicology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands*

Received 13 August 1999; received in revised form 7 October 1999; accepted 2 November 1999

**Abstract**

Theoretical and empirical models can be used to model the migration or separation characteristics in micellar electrokinetic chromatography in order to optimise the resolution. In this paper only empirical models were used, because it is easier and more straightforward to obtain these models. Several empirical approaches for the optimisation of the resolution were compared in order to determine which response should be modelled preferably. The use of models of the effective mobility in combination with average plate numbers proved to be the most suitable approach to optimisation of the resolution, because the relative prediction errors of the models of the effective mobility were a factor of 2–4 smaller than the relative prediction errors of the models of the apparent mobility. Moreover for the least separated peak pair the resolutions based on the models of the apparent and effective mobility showed relative prediction errors that were approximately a factor of 2 smaller than the relative prediction errors of the resolutions based on the models of the resolution and separation factor. The predictions of the separation factor based on the different models generally showed lower prediction errors than the predictions of the corresponding resolutions. Although the relative prediction errors were large, particularly for closely migrating compounds, the empirical approach will probably lead to the optimum separation buffer composition. 

*Keywords:* Resolution; Micellar electrokinetic chromatography; Optimization; Mathematical modelling; Fluvoxamine; Fluvoxketone

## 1. Introduction

In capillary zone electrophoresis (CZE) the migration of charged solutes is induced by the application of an electrical field. Separation is achieved by differences in the electrophoretic mobility of the analytes. Therefore this technique is only useful for the analysis of charged compounds [1]. Micellar electrokinetic chromatography (MEKC) was introduced by Terabe and co-workers [2,3] as a technique for the analysis of neutral compounds. Charged micelles migrate under the influence of an applied electrical field. Separation of neutral analytes is achieved by differences in distribution between the aqueous and micellar phase that migrate at different velocities. MEKC is also applicable to the analysis of charged solutes. These analytes are separated by a combination of differences in both electrophoretic mobility and distribution between the two phases [4].

Various characteristics may be calculated on the

*Corresponding author. Tel.: +31-50-3633-337; fax: +31-50-3637-582.

*E-mail address:* g.j.de.jong@farm.rug.nl (G.J. de Jong)

0021-9673/00/$ – see front matter 
PII: S0021-9673(99)01150-4

basis of electropherograms. Some of these characteristics describe migration like the apparent and effective mobility while others describe separation like the resolution and the separation factor (as defined by Eq. (2)). Optimisation of resolution in MEKC may be achieved by changing the composition of the separation buffer. Variables that may be changed are the pH, the type and concentration of the buffer, the type and amount of modifier and the type and concentration of surfactant. The process of optimisation in MEKC is complicated, because the number of variables affecting the separation is large, the degree of interaction between the variables is high and the effect of a change in the variables is hard to rationalise. In recent years several optimisation strategies were proposed. These can be divided roughly into two categories. Some of the strategies are based on physico–chemical models while others use empirical models [5].

Physico–chemical models can be divided into equations that describe the separation between solutes and equations that describe the migration behaviour of individual solutes. Terabe and co-workers [2,3] introduced an equation that gives the resolution as a function of the selectivity factor, the capacity factor, the migration time of the electroosmotic flow marker ($t_{eof}$) and the migration time of the micelles ($t_{mc}$). This equation indicates that the resolution does not increase steadily with the mean retention factor for the solutes to be separated. Foley et al. [6,7] differentiated this equation with respect to $k$. The maximum resolution was obtained for a mean retention factor, $\bar{k} = \sqrt{t_{mc}/t_{eof}}$. This approach does not focus on the optimisation of a certain well-defined MEKC system, but it provides a general optimum for MEKC. A separation buffer composition should be selected that gives capacity factors in the optimum range. The benefits of this strategy are the limited amount of computation and the general applicability, but the main disadvantage is the possibility of missing the actual optimum of a certain MEKC system. Khaledi and co-workers [8,9] introduced physico–chemical models that describe the migration behaviour of both acidic and basic solutes as a function of the separation buffer composition and physico–chemical constants. Buffer properties like the pH and the surfactant concentration and physico–chemical constants like the critical micelle concentration and a number of equilibrium constants were used. The most prominent advantage of this strategy is the fact that during the process of optimisation physico–chemical characteristics of compounds are obtained. Insight is gained in the mechanism of MEKC. Furthermore determination of these characteristics can be useful in other fields of chemistry. An obvious drawback is the fact that the non-linear regression procedure according to Marquardt [10], which is needed to build these physico–chemical models, is an iterative process. The use of iteration is a time consuming process, and the resulting models depend on the initial estimates of the parameters and on the type of iterative procedure.

A number of authors used empirical models. Jimidar et al. [11] modelled the effective mobility of individual compounds by theoretical and empirical models. The theoretical model was similar to the model used by Khaledi and the empirical model was a second-order polynomial. They used the absolute difference in mobility as a measure of the separation, but did not calculate the resolution. Although the quality of the models was tested by evaluating the residual errors of the training set and the use of four test points, no replicate measurements were performed. Consequently a lack-of-fit test could not be performed and the prediction errors could not be compared to the measurement errors. The empirical models predicted slightly better than the theoretical models. Bütehorn and Pyell [12,13] built linear first degree models for the migration time of the electroosmotic flow marker, the logarithm of the migration time of the micelles and the logarithm of the capacity factors of the solutes. They used these models to calculate the resolution between peaks. Resolutions were calculated using a plate number that was assumed to be constant at various measurement conditions and for all compounds. The limitation of this approach seems that only three measurements were used for the construction of several interrelated models. On the other hand the insight that is gained in the mechanism of MEKC by modelling the migration time of the electroosmotic flow marker and the logarithm of the migration time of the micelles is beneficial. The resolution between adjacent peaks was modelled directly using a second-order polynomial equation by Yik and co-workers [14,15]. First of all it is in principle incorrect to model the

resolution, because it is a complex function that may exhibit discontinuities when peak cross-over occurs. Although peak cross-over did not occur in this case as was established using standard solutions, this approach is not universally applicable. Additionally their models appeared to have better descriptive than predictive quality as the number of terms in the models was equal to the number of measurements. A first-order linear model was used by Vindevogel and Sandra [16] to relate the resolution to a number of variables of the separation buffer. Their approach is useful to determine which variables influence the resolution, but it is questionable whether a first-order model is suitable to fit the resolution for optimisation purposes.

Theoretical models describe the mechanism of MEKC and thus lead to a better understanding of the technique, but this is not necessary for an efficient optimisation. Because the majority of the theoretical models are non-linear, iterative regression methods are needed to fit these models. The results are highly dependent on the initial estimates of the parameters. Therefore, in this paper, emperical models will be investigated. The main objective of this paper is to determine which response should be modelled preferably to enable resolution optimisation in MEKC. The apparent and effective mobility, the separation factor and the resolution are not equally well suitable to be modelled as a function of the separation buffer composition. Characteristics that describe separation like the resolution and the separation factor are complex criteria that may display discontinuous functions in the case of peak cross-over. These discontinuities interfere with the use of polynomial equations in the model building process. In order to overcome the problem of peak cross-over we allow the resolutions and separation factors to become negative and they are calculated not only for adjacent peaks but for all peak pairs. This approach prevents the occurrence of discontinuities and thus enables the modelling of the resolution and the separation factor. Furthermore it assures the correct determination of the minimum resolution as the minimum resolution between adjacent peak pairs equals the minimum resolution of all peak pairs. Our first approach will be to model both the apparent and effective mobility of the various components of the test mixture as a function of two variables (the modifier and surfactant concentration). The models of the apparent and effective mobility will be used in combination with the plate numbers to predict resolutions. A different approach will be to build models for the resolution and the separation factor of the various peak pairs. The models of the separation factor will be employed together with the plate numbers to predict resolutions. The descriptive and predictive quality of the models will be tested by performing replicate measurements and by the use of a test set. This test set will be used to calculate the prediction errors and the replicate measurements will be used to perform a lack-of-fit test and to calculate the measurement error which will be compared to the prediction errors.

## 2. Theory

The resolution ($R_S$) may be expressed in terms of the migration times of the analytes ($t$) and the peak widths at half height ($w_h$):

$$R_S = 1.175 \cdot \frac{t_2 - t_1}{w_{h1} + w_{h2}} \quad (1)$$

Several characteristics that describe separation were compared by Schoenmakers et al. [17,18] including the resolution and the separation factor. The separation factor ($S$) is determined by the migration times of the analytes ($t$) only:

$$S = \frac{t_2 - t_1}{t_1 + t_2} \quad (2)$$

An important benefit of using the separation factor is the fact that an estimate of the peak width is not needed in its calculation. Both the resolution and the separation factor share the disadvantage that they do not take into account peak asymmetry. The separation factor ($S$) may be converted to resolution ($R_S$) by using the average plate number ($\bar{N}$) and applying the following equation [17]:

$$R_S = \frac{1}{2}\sqrt{\bar{N}} \cdot S \quad (3)$$

Various characteristics may be used to describe migration of compounds for instance the migration time and both the apparent and effective mobility. The apparent mobility ($\mu_{app}$) may be expressed as a

function of the total length of the capillary ($l_{tot}$), the effective length of the capillary ($l_{det}$), the applied voltage ($V$) and the migration time of the analyte ($t_R$) [1]:

$$\mu_{app} = \frac{l_{tot} l_{det}}{V t_R} \quad (4)$$

The effective mobility ($\mu_{eff}$) may be derived from the apparent mobility ($\mu_{app}$) and the mobility of the electroosmotic flow marker ($\mu_{eof}$) [1]. The mobility of the electroosmotic flow marker may be calculated according to Eq. (4) by substituting the migration time of the electroosmotic flow marker for the migration time of the analyte ($t_R$):

$$\mu_{eff} = \mu_{app} - \mu_{eof} \quad (5)$$

It is necessary to convert both the apparent and effective mobility to the resolution in order to enable resolution optimisation. This conversion may be performed by using an equation that was introduced by Giddings [19]. The resolution ($R_S$) is expressed in terms of the average plate number ($N$) and either the apparent mobility ($\mu_{app}$) or the effective mobility ($\mu_{eff}$) and the mobility of the electroosmotic flow marker ($\mu_{eof}$):

$$R_S = \frac{1}{4}\sqrt{N} \cdot \frac{\Delta\mu_{app}}{\bar{\mu}_{app}} = \frac{1}{4}\sqrt{N} \cdot \frac{\Delta\mu_{eff}}{\bar{\mu}_{eff} + \mu_{eof}} \quad (6)$$

A modification of the equation of Giddings may be used to convert both the apparent and effective mobility to the separation factor. This conversion is not necessary to enable resolution optimisation, but it is useful to compare the resolution and the separation factor in terms of measurement error and prediction error. The separation factor ($S$) may be calculated from either the apparent mobility ($\mu_{app}$) or the effective mobility ($\mu_{eff}$) and the mobility of the electroosmotic flow marker ($\mu_{eof}$):

$$S = \frac{1}{2} \cdot \frac{\Delta\mu_{app}}{\bar{\mu}_{app}} = \frac{1}{2} \cdot \frac{\Delta\mu_{eff}}{\bar{\mu}_{eff} + \mu_{eof}} \quad (7)$$

Resolution, separation factor, apparent mobility and effective mobility will be modelled to predict the resolution. It is difficult to decide which terms and cross-terms should be used to build a model. A good starting point to construct empirical models for two variables is the full second-order polynomial equation:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (8)$$

A number of diagnostic criteria are available to evaluate the performance of a model. The most widely used descriptive criterion is the correlation coefficient ($R^2$). It is calculated from the regression sum of squares ($SS_{reg}$) and the total sum of squares ($SS_{tot}$). The average response ($\bar{y}$) and both the measured ($y_i$) and the predicted ($\hat{y}_i$) response of the individual training points ($n$) are necessary to calculate these sums of squares:

$$R^2 = \frac{SS_{reg}}{SS_{tot}} = \frac{\sum_{i=1}^{n} (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \quad (9)$$

A useful predictive criterion is the relative mean squared error of prediction [20]. It is necessary to have a set of data that are not used to build the model, i.e., the test points. The relative mean squared error of prediction ($MSEP_{rel}$) is calculated using the average response ($\bar{y}$) and both the measured ($y_k$) and the predicted ($\hat{y}_k$) responses of all the test points ($m$):

$$MSEP_{rel} = \frac{\sqrt{\frac{1}{m} \cdot \sum_{k=1}^{m} (y_k - \hat{y}_k)^2}}{\bar{y}} \cdot 100\% \quad (10)$$

The relative mean squared error of prediction has to be compared to some value that represents the amount of measurement variation in the data. This is necessary to evaluate the quality of the model: models will perform well if their error of prediction is comparable to the measurement error of the data. The relative standard deviation based on replicate measurements (RSD) is suitable for this purpose. It may be obtained by introducing the measured response ($y_{ij}$) of the individual replicated training points ($n$), the average response ($\bar{y}_i$) for each training point, the number of replicates ($n_i$) for each training point and the overall average response ($\bar{y}$) into the following equation with subscript $i$ denoting the training point and subscript $j$ denoting the replicate:

$$\mathrm{RSD} = \frac{\sqrt{\dfrac{\sum_{i=1}^{n}\sum_{j=1}^{n_i}(y_{ij}-\bar{y}_i)^2}{\sum_{i=1}^{n}(n_i-1)}}}{\bar{y}} \cdot 100\% \qquad (11)$$

## 3. Experimental

### 3.1. Chemicals

The antidepressant fluvoxamine (*E* isomer) and three related compounds, i.e., the *Z* isomer (Z isomer), an addition product (adduct) and fluvoxketone (ketone) were donated by Solvay Pharmaceuticals (Weesp, The Netherlands) (Fig. 1). Acetonitrile was obtained from Labscan (Dublin, Ireland) and acetone (the electroosmotic flow marker), boric acid, sodium dodecyl sulphate (SDS) and sodium hydroxide from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Deionised water was prepared using an Elga Maxima ultra pure water system (Salm & Kipp, Breukelen, The Netherlands). A solution of the test mixture was prepared in water containing $0.71 \cdot 10^{-4}$ *M* of the *E* isomer, $1.3 \cdot 10^{-4}$ *M* of the *Z* isomer and $2.0 \cdot 10^{-4}$ *M* of both the adduct and the ketone. Borate buffer (25 m*M*, pH 9.3) was prepared as background electrolyte. The buffer was adjusted to the proper pH by addition of 2.0 *M* sodium hydroxide. Varying amounts of acetonitrile and SDS were added to the buffer. The separation buffer was filtered through a 0.45-μm membrane filter (Schleicher & Schuell, Dassel, Germany) before use.

### 3.2. CE system

Experiments were carried out on a Hewlett-Packard 3D capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany), equipped with a diode-array detection system and ChemStation Release 04.02 for system control, data acquisition and data analysis. The samples were injected hydrodynamically by applying a pressure of 50 mbar for 2 s. Electrophoresis was performed at a constant voltage of 30 kV and the temperature of the capillary was maintained at 30°C. An uncoated fused-silica capillary of 62.0 cm (effective length 54.0 cm)×50 μm I.D. was used. Before use the capillary was rinsed with 1 *M* sodium hydroxide (15 min), followed by deionised water (15 min) and separation buffer (30 min). Between runs the capillary was flushed with the buffer for 2 min. After change of the separation buffer the capillary was flushed with the new buffer for 30 min.

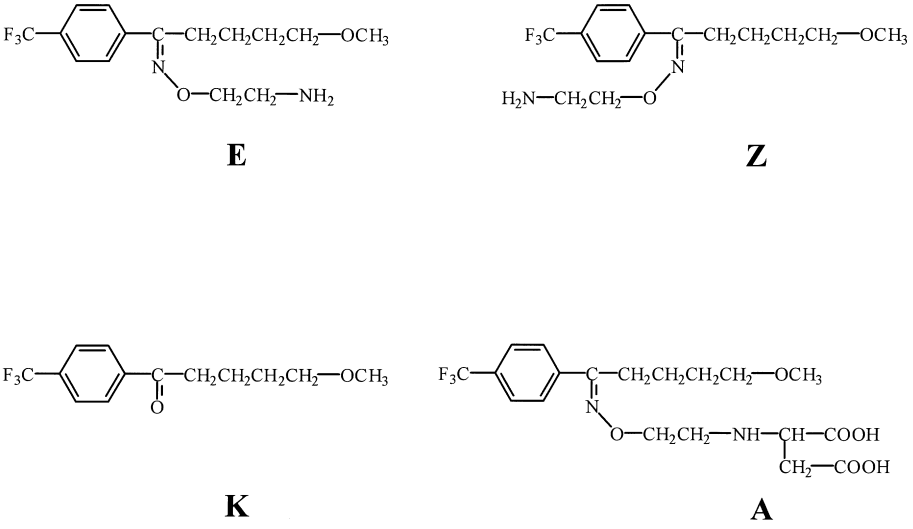


Fig. 1. The structures of fluvoxamine (E), the *Z* isomer (Z), fluvoxketone (K), and fluvoxamine adduct (A).

### *3.3. Experimental design and software*

The resolution, the separation factor, the apparent mobility and the effective mobility were related to two experimental factors, the percentage of acetonitrile and the concentration of SDS, by normal least-squares regression. The factor space was limited by an acetonitrile content in the range 0–15% and a concentration of SDS in the range 25–100 m*M*. A total of 14 training points and four test points were selected in this factor space (Fig. 2). Experiments were carried out using these buffer compositions. Each experiment was performed three to six times. The training points were used to build models and the test points were used to evaluate the predictive quality of the models. All calculations were performed using spreadsheets in Microsoft Excel 97. The coefficients of the models were obtained using regression routines programmed in Matlab 4.2c (MathWorks, Natick, MA, USA).

## 4. Results and discussion

### *4.1. Determination of migration and separation characteristics*

The *Z* isomer, addition product and fluvoxketone were selected as components of the test mixture, because these compounds are possible impurities of the antidepressant fluvoxamine (*E* isomer). Both the *Z* and *E* isomers are primary amines with a p$K_a$ value of 9.3, the addition product contains acidic and basic groups and fluvoxketone is a neutral compound. The separation of this mixture by CZE and MEKC was compared and evaluated by Hilhorst et

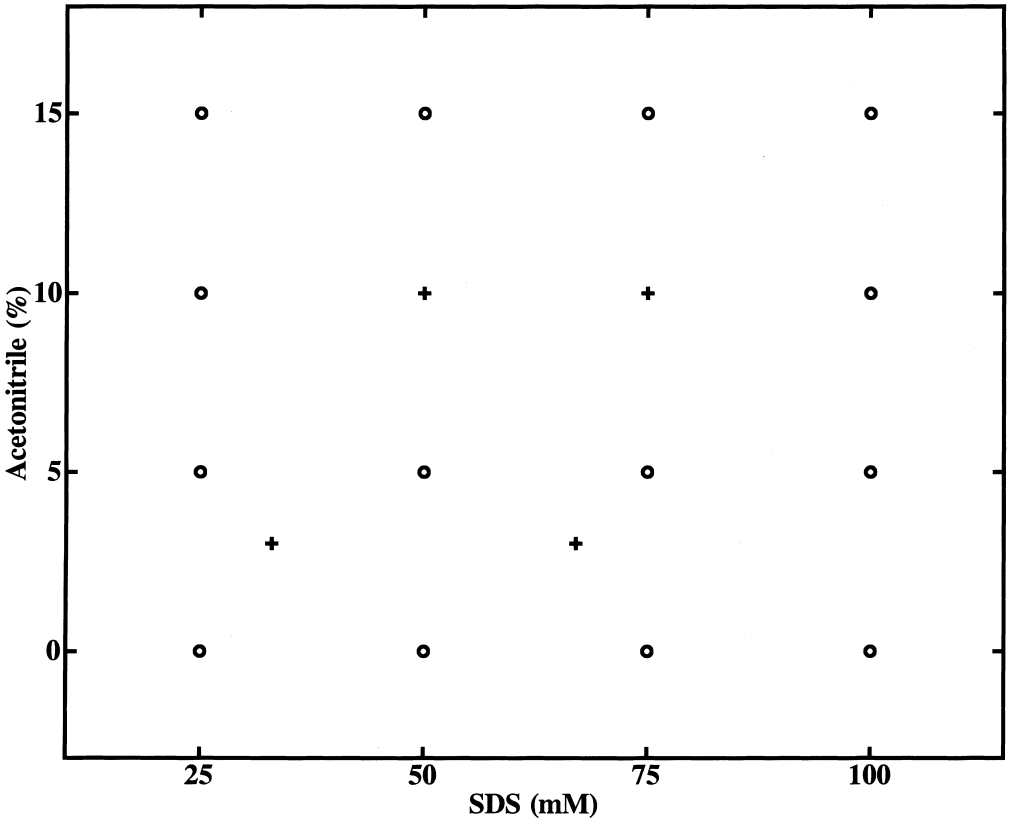


Fig. 2. Factor space of the MEKC system for the separation of fluvoxamine and related compounds. Training points (○) were used to build models, while test points (+) were used to validate the models.

al. [21]. In the present study electropherograms were obtained according to the experimental design (Fig. 2). An electropherogram obtained with a separation buffer containing 10% of acetonitrile and 50 m*M* of SDS showed relatively high resolutions (Fig. 3). Resolutions and separation factors were calculated according to Eqs. (1) and (2) and the apparent and effective mobilities according to Eqs. (4) and (5), respectively.

### 4.2. Modelling

The apparent and effective mobilities were modelled as a function of the concentration of acetonitrile and SDS in the separation buffer. A full second-order polynomial was used according to Eq. (8). In order to judge the descriptive and predictive quality of the models the correlation coefficients were calculated according to Eq. (9) (Table 1) and the relative mean squared errors of prediction according to Eq. (10) (Table 1). The correlation coefficients of the models of the apparent mobility were approximately equal for the different compounds, i.e., in the range 0.98–0.99, whereas the correlation coefficients of the models of the effective mobility decreased

Table 1
Correlation coefficients and relative MSEPs of the apparent and effective mobility models

| Component | Correlation coefficient | | Relative MSEP (%) | |
|---|---|---|---|---|
| | Apparent mobility | Effective mobility | Apparent mobility | Effective mobility |
| Adduct | 0.99 | 0.95 | 6.5 | 3.9 |
| Ketone | 0.98 | 0.90 | 11 | 2.5 |
| *Z* isomer | 0.99 | 0.67 | 12 | 2.8 |
| *E* isomer | 0.98 | 0.57 | 12 | 2.9 |

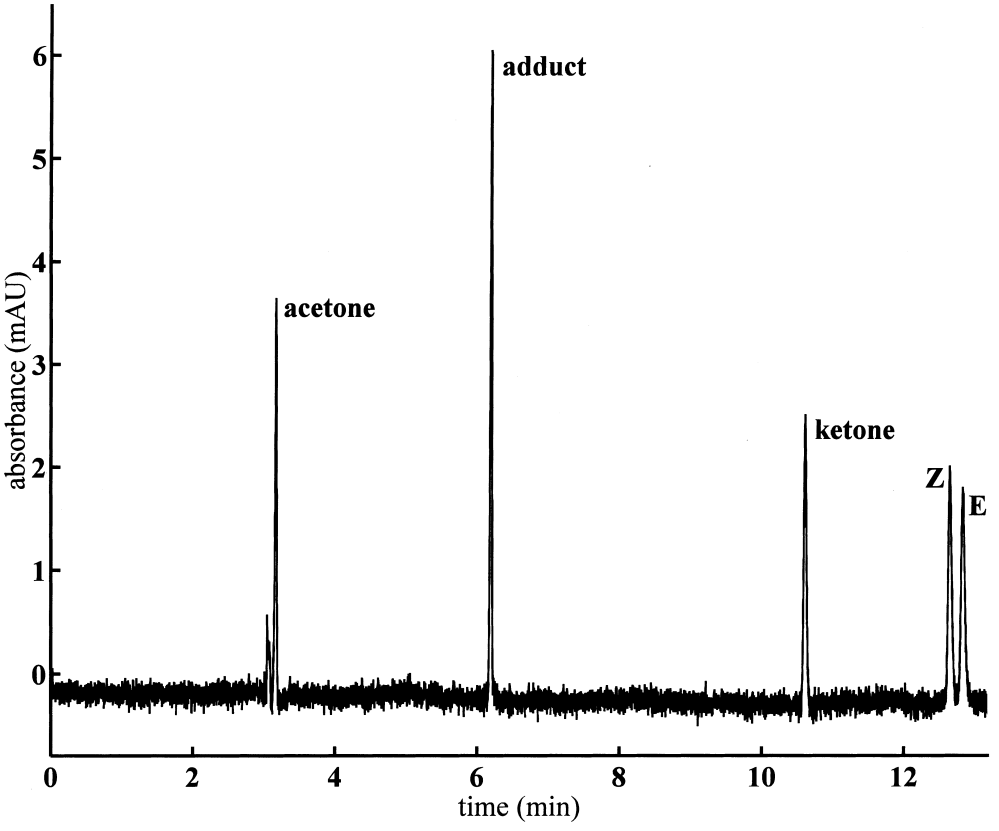


Fig. 3. Electropherogram of fluvoxamine (*E* isomer) and related compounds. The borate buffer (pH 9.3) contains 10% acetonitrile and 50 m*M* SDS.

from 0.98 for the ketone down to 0.57 for the *E* isomer. However the relative mean squared errors of prediction were higher for the models of the apparent mobility (about 7% for the adduct and about 12% for the other compounds) than for the models of the effective mobility (approximately 3%).

From a theoretical point of view modelling of effective mobilities should lead to higher correlation coefficients and lower relative prediction errors than modelling of apparent mobilities. Effective mobilities should have a more straightforward relation to the separation buffer composition, because they depend on the physico–chemical properties of compounds, whereas apparent mobilities are also dependent on the properties of the capillary surface due to a contribution of the electroosmotic flow. In practice the relative mean squared errors of prediction of the models of the effective mobility proved to be lower indeed, but surprisingly the correlation coefficients were also lower. A possible explanation for the higher correlation coefficients of the apparent mobilities is the fact that in MEKC both the migration time of the micelles and the migration times of the compounds are highly correlated to the migration time of the electroosmotic flow marker. Therefore the apparent mobilities are also well correlated to the mobility of the electroosmotic flow marker which exhibits a very strong correlation to the separation buffer composition (correlation coefficient is approximately 0.98). Nevertheless modelling of effective mobilities is preferred because the models of the apparent mobility displayed higher prediction errors.

The relatively low correlation coefficients for the models of the effective mobility of the *Z* and *E* isomers can be explained by two effects. Firstly, the *Z* and *E* isomers had a high affinity for the micelles and, therefore, a change in the separation buffer composition did not cause a considerable change in the effective mobilities of these compounds. Secondly, the combination of Eqs. (4) and (5) indicates that the variation of the effective mobility will be lower, if the migration time of a compound is large compared to the migration time of the electroosmotic flow marker (Eq. (12)). For $\mu_{\text{eff}}$ the following equation can be given:

$$\mu_{\text{eff}} = \frac{l_{\text{tot}} l_{\text{det}}}{V} \cdot \frac{t_{\text{eof}} - t_{\text{R}}}{t_{\text{R}} t_{\text{eof}}} \qquad (12)$$

This would also lead to low correlation coefficients for the models of the *Z* and *E* isomers, because these compounds were late migrating.

In order to be able to compare the different approaches for optimisation the resolution and separation factor were modelled according to Eq. (8). The correlation coefficients of these models are not shown, because they were only built to compare their predictive quality with that of the models of the apparent and effective mobility. In order to judge the predictive quality of the models the relative mean squared errors of prediction were determined according to Eq. (10). An attempt was made to model the plate numbers of the various compounds as a function of the separation buffer composition according to Eq. (8). These efforts proved to be useless as the correlation coefficients turned out to be in the order of 0.00–0.10. Consequently the plate numbers were assumed to be independent of the separation buffer composition and it was decided to use the average plate numbers. The average plate numbers were approximately $3.7 \cdot 10^5$ for the adduct, $3.2 \cdot 10^5$ for the ketone, $2.7 \cdot 10^5$ for the *Z* isomer and $2.3 \cdot 10^5$ for the *E* isomer. It should be mentioned that the variation in the plate numbers was very large. The standard deviations of the plate numbers were $1.7 \cdot 10^5$ for the adduct, $2.1 \cdot 10^5$ for the ketone, $2.1 \cdot 10^5$ for the *Z* isomer and $1.6 \cdot 10^5$ for the *E* isomer. This large variation could have masked a dependence of the plate number on the separation buffer composition. It is very unlikely that the large variation in the peak width is caused by the data system. Firstly, the applied data system was designed to process the very small peaks in capillary electrophoresis. Secondly, the variation in the peak width is not completely random: if one peak in an electropherogram is wider, the other peaks in the same electropherogram are also wider. This observation indicates that the problem originates from the MEKC system.

### *4.3. Prediction of the resolution*

#### *4.3.1. Comparison of the response surfaces*

The resolution between the peak pair of the *Z* and *E* isomers is equal to the minimum resolution over the complete factor space. Therefore the response surfaces of the resolution of this peak pair are shown

based on the models of the resolution (Fig. 4a), the separation factor (Fig. 4b), the apparent mobility (Fig. 4c) and the effective mobility (Fig. 4d). The measured resolutions for a separation buffer containing 25 m*M* of SDS and 15% of acetonitrile show considerable variation. Moreover the measurements

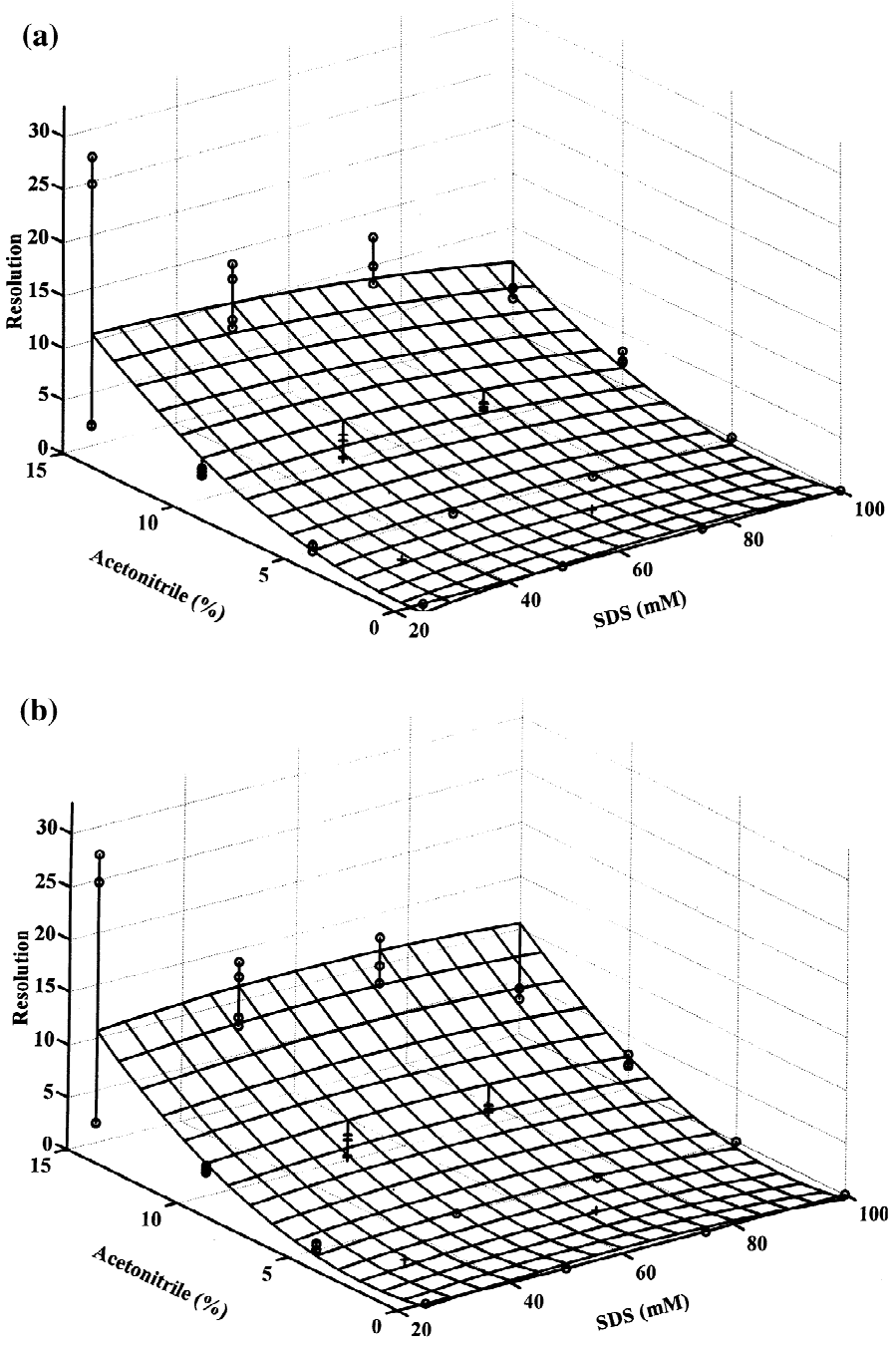


Fig. 4. Response surfaces for the resolution of the least separated peak pair (*Z* and *E* isomers) based on the models of the resolution (a), separation factor (b), apparent mobility (c) and effective mobility (d). Both the training points (○) and test points (+) were included.

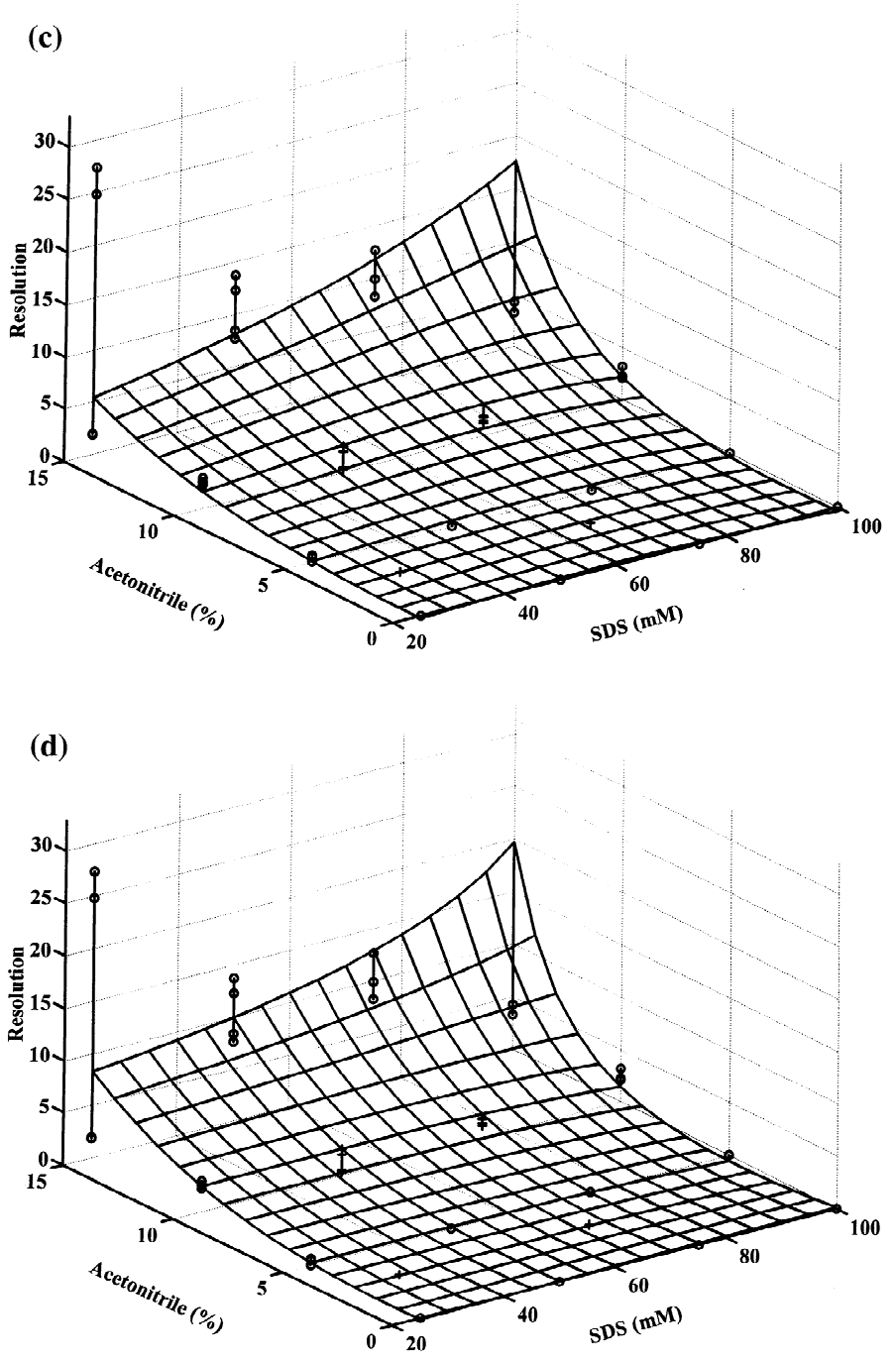


Fig. 4. (*continued*)

at this separation buffer composition proved to be outliers and were not used to build any of the models.

The response surfaces based on the models of the resolution and separation factor appear to be quite similar. Moreover the response surfaces based on the

models of the apparent and effective mobility look also similar. However the response surfaces based on the models of the resolution and separation factor on the one hand and those based on the models of the apparent and effective mobility on the other hand appear to differ substantially at simultaneously high concentrations of acetonitrile and SDS. The models of the resolution and separation factor fit more closely to these training points than the models of the apparent and effective mobility. But then the measurements under these conditions (separation buffer containing 100 m*M* SDS and 15% acetonitrile) were not reliable as in two out of four electropherograms the peaks of the *E* and the *Z* isomer could not be recovered at all. The models of the resolution and separation factor are influenced strongly by one unreliable training point, while this is not true for the models of the apparent and effective mobility. This difference may be explained by the fact that in the case of the resolution and separation factor one model is used to predict a resolution and in the case of the apparent or effective mobilities two models are used to calculate a resolution.

### *4.3.2. Comparison of the prediction errors*

The relative mean squared errors of prediction are shown for the resolutions based on the models of the resolution, the separation factor and both the apparent and effective mobility (Fig. 5). These prediction errors were calculated according to Eq. (10). RSDs of the data are also shown. The prediction errors of the resolutions based on the different models are approximately equal except for the peak pair of the *Z* and *E* isomers. For this peak pair the prediction errors based on the models of the apparent and effective mobility are better than those based on the models of the resolution and separation factor. Besides the prediction errors of the resolutions based on the different models are roughly the same as the corresponding RSD apart from the peak pair of the ketone and the *E* isomer which shows slightly higher prediction errors and the peak pair of the *Z* and *E*

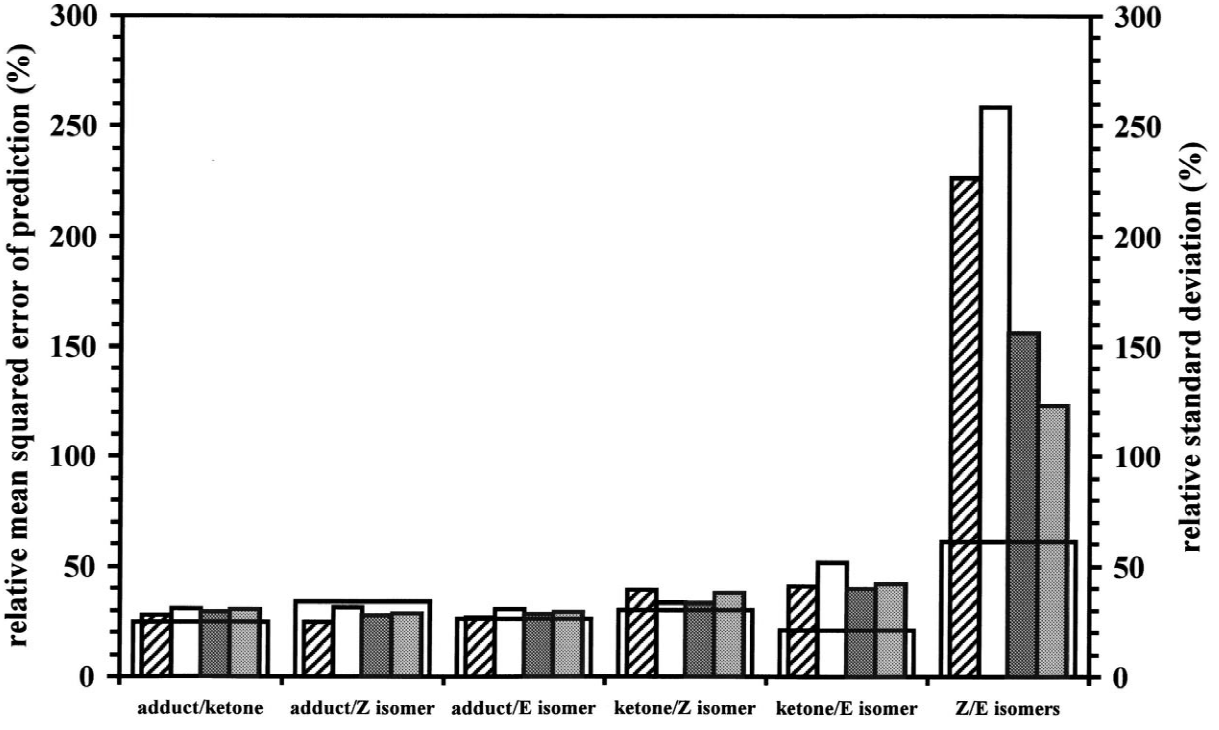


Fig. 5. Relative MSEP of the resolution based on the models of the resolution (*hatched*), the separation factor (*white*), the apparent mobility (*dark gray*) and the effective mobility (*light gray*). The RSD is indicated by the overlay.

isomers which displays much higher prediction errors.

The relative mean squared errors of prediction are also shown for the separation factors based on the models of the separation factor and both the apparent and effective mobility (Fig. 6). The figure also includes the RSD of the data. The prediction errors of the separation factor based on the different models are about equal except for the peak pair of the *Z* and *E* isomers which exhibits a higher prediction error based on the model of the separation factor than based on the models of the apparent and effective mobility. Moreover the prediction errors of the separation factors based on the different models are roughly the same as the corresponding RSDs apart from the peak pairs of the ketone and both the *Z* and the *E* isomers which show slightly higher prediction errors and the peak pair of the *Z* and *E* isomers which displays much higher prediction errors.

It should be noted that the prediction errors as well as the coefficients of variation are considerably lower for the separation factor than for the resolution except for the peak pair of the *Z* and *E* isomers. As the only difference between the resolution (Eq. (6)) and the separation factor (Eq. (7)) is the plate number, the variation in the peak width has to be responsible for a major part of the measurement and prediction error of the resolution. However for the peak pair of the *E* and *Z* isomers both the prediction errors and the coefficients of variation are equal for the separation factor and the resolution. As the prediction errors based on the models of the resolution and separation factor are higher than those based on the models of the apparent and effective mobility, the problem of modelling a composed criterion like the resolution or the separation factor has to be responsible for at least a part of this prediction error.

## 5. Conclusions

Models of the effective mobility in combination with average plate numbers appear to be the most

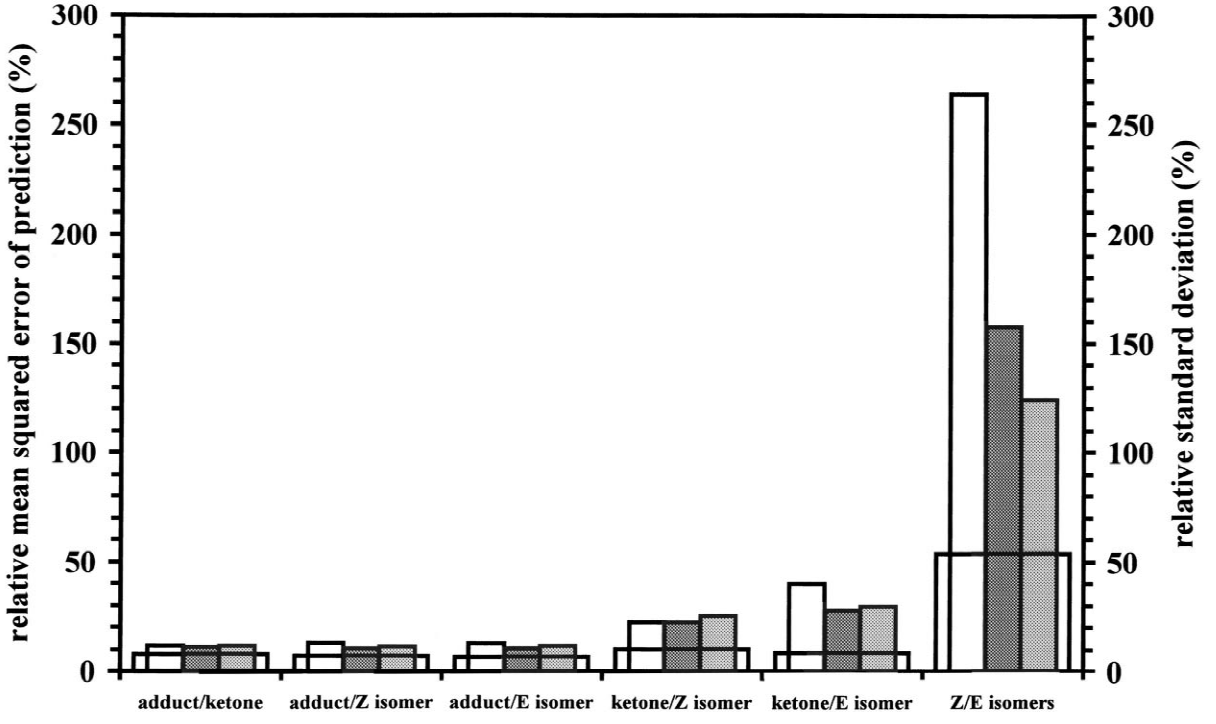


Fig. 6. Relative MSEP of the separation factor based on the models of the separation factor (*white*), the apparent mobility (*dark gray*) and the effective mobility (*light gray*). The RSD is indicated by the overlay.

suitable approach for resolution optimisation. In the first place models of the effective mobility display relative prediction errors that are approximately a factor of 2–4 smaller than the relative prediction errors of the corresponding models of the apparent mobility. Secondly for the least separated peak pair (that of the *Z* and the *E* isomers) the resolutions based on the models of the apparent and effective mobility show relative prediction errors that are a factor of 2 lower than the relative prediction errors of the resolutions based on the models of the resolution and separation factor. The limitation of this approach is that the variation in the peak width is large in MEKC. It proved to be impossible to model the plate number as a function of the separation buffer composition, because the amount of random variation was substantially higher than the amount of variation that correlated to the separation buffer composition. Furthermore the RSDs of the resolutions were approximately a factor of 1.5–3 larger than the RSDs of the corresponding separation factors except for the least separated peak pair. Although the prediction errors of the resolution are generally high, especially for closely migrating compounds, the results of the optimisation procedure are useful. The variances in the migration of compounds are usually correlated as was shown by Wieling et al. [22]. This means that although the predicted optimum resolution may not be quantitatively correct, the optimisation procedure will probably lead to the separation buffer composition that gives the best possible resolution in the factor space. In future experiments the emphasis may be on the improvement of the stability of the analytical system, because this might enhance the possibility to model the plate number for the separate compounds as a function of the separation buffer composition.

## References

[1] J.W. Jorgenson, K.D. Lukacs, J. High Resolut. Chromatogr. Chromatogr. Commun. 4 (1981) 230.
[2] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[3] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[4] J. Vindevogel, P. Sandra, Introduction to Micellar Eletrokinetic Chromatography, Hüthig, Heidelberg, 1992.
[5] H. Corstjens, H.A.H. Billiet, J. Frank, K.Ch.A.M. Luyben, J. Chromatogr. A 715 (1995) 1.
[6] J.P. Foley, Anal. Chem. 62 (1990) 1302.
[7] K. Ghowsi, J.P. Foley, R.J. Gale, Anal. Chem. 62 (1990) 2714.
[8] M.G. Khaledi, S.C. Smith, J.K. Strasters, Anal. Chem. 63 (1991) 1820.
[9] C. Quang, J.K. Strasters, M.G. Khaledi, Anal. Chem. 66 (1994) 1646.
[10] D.W. Marquardt, J. Soc. Ind. Appl. Math. 11 (1963) 431–441.
[11] M. Jimidar, B. Bourguignon, D.L. Massart, Anal. Chim. Acta 310 (1995) 27.
[12] U. Pyell, U. Bütehorn, J. Chromatogr. A 716 (1995) 81.
[13] U. Bütehorn, U. Pyell, J. Chromatogr. A 772 (1997) 27.
[14] Y.F. Yik, S.F.Y. Li, Chromatographia 35 (1993) 560.
[15] Y.J. Yao, H.K. Lee, S.F.Y. Li, J. Chromatogr. 637 (1993) 195.
[16] J. Vindevogel, P. Sandra, Anal. Chem. 63 (1991) 1530.
[17] P.J. Schoenmakers, Optimization of Chromatographic Selectivity, Elsevier, Amsterdam, 1986.
[18] P.F. Vanbel, B.L. Tilquin, P.J. Schoenmakers, Chemom. Intell. Lab. Sys. 35 (1996) 67.
[19] J.C. Giddings, Sep. Sci. 4 (1969) 181.
[20] D.A. Harville, D.R. Jeske, J. Am. Stat. Assoc. 87 (1992) 724.
[21] M.J. Hilhorst, G.W. Somsen, G.J. de Jong, J. Pharm. Biomed. Anal. 16 (1998) 1251–1260.
[22] J. Wieling, P.M.J. Coenegracht, D.A. Doornbos, J.H.G. Jonkman, J. Chromatogr. 635 (1993) 195.