Optimization of resolution in micellar electrokinetic chromatography via computer-aided variation of concentrations of sodium dodecyl sulfate and acetonitrile as modifier

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Abstract

A computer-aided method is presented, that permits optimizing simultaneously the concentrations of an anionic surfactant (sodium dodecyl sulfate) and the modifier acetonitrile in the separation buffer in micellar electrokinetic chromatography (MEKC) on the basis of four test runs. The presented method was developed for the separation of non-polar solutes. The underlying algorithm takes into account that resolution in MEKC is dependent on selectivity, retention factor and the ratio hold-up time/migration time of the micelles (t_0/t_M). It is shown that with high surfactant concentrations and high acetonitrile concentrations a nearly infinite elution range is approached. The presented optimization method is applied to the separation of biogenic amines derivatized with dansyl chloride. It is shown that under the conditions usually employed in MEKC up to a volume fraction of acetonitrile in the separation buffer of 0.20 the critical micellar concentration of the surfactant investigated is increased only to a small extent.

Keywords: Computer-assisted optimization; Optimization; Buffer composition; Amines

1. Introduction

Micellar electrokinetic chromatography (MEKC), first introduced by Terabe et al. [1,2], renders possible the separation of neutral and charged solutes by distributing them between an aqueous mobile phase and a retarded micellar phase (pseudo-stationary phase). The versatility and high efficiency of this method and its potential importance in routine analysis make it desirable that rapid and effective means of the optimization of the separation buffer are available. The optimization of separations performed with MEKC is complex and difficult due to the high number of parameters affecting the separation [3]. Further complications can arise from the mutual interaction of the parameters to be optimized.

Computer-assisted approaches have proven suitable to meet the requirements for rapid and reliable resolution optimization in MEKC rather than optimization by trial and error. Corstjens et al. [3] have recently given an overview on statistical approaches in the optimization of separations in capillary electrophoresis (CE) and especially MEKC and on optimization procedures based on physicochemical models. All procedures have in common that they provide guidelines to achieve an adequate selectivity with a minimum number of experiments. Vindevogel and Sandra [4] used a Plackett–Burman statistical design to optimize the resolution of testosterone esters. Yik and Li [5] used a three-dimensional overlapping resolution mapping scheme to optimize the pH, the micelle concentration and the concentration of an ion-pair reagent. In their statistical approach, eleven initial experiments are afforded.

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Smith and Khaledi [6] predicted the migration behavior of an homologous series of phenols over a two-dimensional pH/micelle concentration space employing physicochemical constants, that have to be determined prior to the optimization procedure. Quang et al. [7] predicted successfully the migration behavior of several acidic and basic solutes over a pH–micelle concentration factor space on the basis of only five experiments. Corstjens et al. [8] employed the iterative regression strategy for the simultaneous optimization of the pH and the micelle concentration. Only seven experiments were required. Wiedmer et al. [9] optimized the selectivity in MEKC with a mixed micellar system (sodium dodecyl sulfate and sodium cholate as surfactants). In this study four parameters were taken into account requiring 25 initial experiments.

The computer-assisted optimization procedures developed differ largely concerning the number of test runs required, the parameters optimized (i.e surfactant concentration, pH, modifier concentration) and the algorithm underlying. Versatility, low number of test runs and matching of predicted optimum to experimental data will be the decisive parameters for the feasibility of these strategies in method development. Pre-selection of parameters and the parameter space to be optimized, a model or algorithm to describe the migration behavior of the solutes and a criterion to evaluate the resulting chromatograms are indispensable for this purpose.

In recent studies, Pyell and Bütehorn [10] have shown that for moderately polar solutes effective resolution optimization can be obtained if the parameters to be varied are the concentration of the anionic surfactant (sodium dodecyl sulfate, SDS) and the modifier (urea) in the separation buffer. They predicted the optimum buffer composition on the basis of only three test runs employing the basic equation for resolution (R_s) of two adjacent peaks developed by Terabe et al. [1,2]:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha-1}{\alpha} \cdot \frac{1-t_0/t_M}{1+t_0/t_M \cdot k} \qquad (1)$$

where: $N =$ Plate number; $k =$ mean retention factor; $\alpha =$ selectivity factor; $t_0 =$ migration time of the mobile phase; $t_M =$ migration time of the micelles.

Terabe et al. [2] have already shown that the function $f(k) = (k/(1+k)) \cdot (1-t_0/t_M)/(1+(k \cdot t_0/t_M))$

passes through a maximum, if t_M is not infinite. Foley [11] demonstrated that the basic equation for resolution in MEKC differentiated for k has a zero value if $k = \sqrt{t_M/t_0}$, provided that the other quantities in Eq. (1) are kept constant. This assumption, however, is not fulfilled in practice: the retention factors can be influenced by changing the phase ratio (pseudostationary phase/mobile phase) by variation of the surfactant concentration or they can be decreased by addition of a modifier, thus decreasing the distribution constants of the solutes to be separated. However, in both cases the ratio t_0/t_M and possibly α are also affected making resolution optimization to a complex problem [3,12,13].

In the approach of Pyell and Bütehorn urea was selected as modifier, because it was reported [14,15] to be a versatile modifying additive to the mobile phase that greatly improved the ratio t_0/t_M without reducing strongly the electroosmotic velocity, v_{eo}. The addition of urea to the mobile phase also decreased the retention factors of the solutes, thus making it possible to adjust them to the optimum condition derived by Foley [11]. If the solutes to be separated are non-polar, however, the addition of urea to the separation buffer does not decrease the retention factors for the solutes sufficiently, so that they come into the optimum range. Therefore, the approach of Pyell and Bütehorn [10], developed for moderately polar solutes, is not applicable with non-polar solutes. In the case of non-polar solutes a modifier has to be selected that has a larger impact on the retention factors for the solutes than urea.

In the normal elution mode for MEKC, the ideal organic modifier has the following features: (i) it does not influence greatly the CMC of the surfactant (ii) it decreases k and t_0/t_M (iii) it does not prolong unnecessarily the run time by increasing t_0. Comparing the desired features with literature measurements, acetonitrile was selected as organic modifier for the separation of non-polar solutes. Acetonitrile has been reported to be an effective modifier in MEKC separations [12,16–20] reducing the retention factors more effectively than urea and decreasing t_0/t_M, thus enlarging the elution window. Schwer and Kenndler [21] have demonstrated that the addition of acetonitrile to the separation buffer increases t_0 to a lesser extent than alcoholic modifiers or dimethylsulfoxide.

As part of our study the CMC of SDS dependent on the volume fraction of acetonitrile was ex-

perimentally determined under conditions usually employed in MEKC in order to verify that the addition of acetonitrile to the separation electrolyte does not affect greatly the phase ratio (volume of pseudostationary phase/volume of mobile phase; V_s/V_m).

In this paper, a computer-aided method based on a computer program called Computer Assisted Bivariate Resolution Optimization II (CABRO II) is presented that permits the prediction of the optimum electrolyte composition for separations of non-polar solutes performed with MEKC. The optimized parameters are the concentration of the surfactant sodium dodecyl sulfate and the modifier acetonitrile on the basis of only four test runs. The pH of the separation electrolyte is not varied. Changes of the apparent selectivities, the elution order, the retention factors and t_0/t_M in dependence on the electrolyte composition are taken into consideration in the algorithm. CABRO II was employed for the optimization of the separation of a mixture of 14 biogenic amines derivatized with dansyl chloride. Heptylamine is employed as internal standard [22].

2. Experimental

2.1. Reagents

Most of the amines employed as standards were available at the Department of Chemistry (University of Marburg). 1,4-Diaminobutane, 3-methylbutylamine, 1,5-diaminopentane, hexylamine and heptylamine were purchased from Aldrich (Steinheim, Germany). Sudan III was from Fluka (Buchs, Switzerland).

$Na_2HPO_4 \cdot 12 \ H_2O$, sodium tetraborate, boric acid (Merck, Darmstadt, Germany) and SDS (Roth, Karlsruhe, Germany) used for the preparation of the separation electrolytes and dansyl chloride [5-(dimethylamino)-naphthalin-1-sulfonylchloride (Aldrich)] were of analytical grade. Acetonitrile was distilled. Water was twice distilled.

2.2. Determination of the CMC

The CMC of SDS in buffers with different volume fraction of acetonitrile at 30°C were determined conductometrically and photometrically. Buffers

contained 6.25 mM Na_2HPO_4 and 1.25 mM $Na_2B_4O_7$ in a solution of acetonitrile in water. Buffer solutions with varying concentrations of SDS were prepared by the dilution of appropriate quantities of an SDS stock solution in buffer with pure buffer.

In the case of a conductometric determination of the CMC the conductivity of the buffer in dependence on the SDS concentration was determined with a model E 365 B conductoscope (Metrohm, Herisau, Switzerland) and two platinum wire electrodes. The temperature of the titration vessel was controlled with a water jacket.

In the case of a photometric determination of the CMC, anthracene was added in excess to buffer solutions with varying concentrations of SDS. The vials were then sealed with PTFE-lined silicone caps. The solutions were saturated with anthracene by agitating overnight in a shaker equipped with a thermoregulated water bath. After saturation the excess anthracene was rapidly filtered off. Absorbance measurements of the solutions saturated with anthracene were made at a wavelength of 254 nm with a Hitachi (Tokyo, Japan) U-3410 spectrophotometer equipped with a thermoregulated cell holder, using quartz cells of 1-cm path length.

2.3. Derivatization of amines

10–20 µl of a saturated solution of dansyl chloride in acetone were given to a solution of amine in 10 ml borate buffer ($c_{Na_2B_4O_7} = 10$ mM; $c_{SDS} = 20$ mM; pH 9) dissolved in 10 ml acetone. The solution was heated to 70°C in a closed vessel for 30 min. After completion of the derivatization the solution was poured into a beaker and the acetone was evaporated by heating the solution to 70°C for about 15 min.

2.4. Chromatographic measurements

All chromatographic measurements were carried out with a Beckman (Fullerton, CA, USA) model P/ACE capillary electrophoresis system equipped with a UV-absorbance detector. The temperature of the capillary was controlled by liquid cooling and was maintained at 25°C. Samples were injected by application of pressure for 2 s. Detection was performed at 254 nm. All separations were carried

out at a voltage of 25 kV. Data were recorded with the Beckman System Gold software.

Fused-silica capillaries (75 μm I.D. × 375 μm O.D.) were obtained from Polymicro Technologies, Phoenix, AZ, USA. The total length of the capillary was 56.5 cm and the length to the detector was 50 cm. The elution time of the mobile phase, t_0, and the elution time of the micellar phase, t_M, were determined using thiourea and sudan III, respectively, as markers. Peak identities were confirmed by spiking.

Studies with varied concentrations of SDS and acetonitrile were performed with a buffer containing 10 mM H_3BO_3 and 10 mM $Na_2B_4O_7$.

2.5. Software

Correlation studies and fitting were performed with MATHEMATICA 2.0 for Windows. All programs used for optimization studies were written in PASCAL employing Turbo Pascal 6.0 (Borland International, CA, USA).

3. Results and discussion

3.1. The influence of the volume fraction of acetonitrile on the CMC

In the literature [2] the phase ratio V_s/V_m is calculated from the CMC, the total concentration of the surfactant and the partial molar volume of the micelles. Determination of the CMC in dependence on the volume fraction of acetonitrile, φ_A, permits evaluation of alterations in V_s/V_m in dependence on φ_A. It is known that the CMC of a surfactant is strongly dependent on the ionic strength. For comparison with results in MEKC it is necessary to determine the CMC under conditions that are usually employed in MEKC. All measurements were therefore performed with buffers containing 6.25 mM Na_2HPO_4 and 1.25 mM $Na_2B_4O_7$ in a solution of acetonitrile in water at a temperature of 30°C.

With conductometric titration, the CMC of the surfactant can be evaluated from the discontinuity of the titration curve. Above the CMC the increase of the conductivity with increasing SDS concentration (c_{SDS}) is lower than below the CMC, because the

formed micelles have a lower electrophoretic mobility than the monomers. It is interesting to note that the difference in the slopes of the titration curve below and above of the CMC is reduced with increasing volume fraction of acetonitrile (see Fig. 1). At $\varphi_A = 15\%$ the CMC cannot be determined with this method, because no discontinuity is observed. These results suggest that at higher φ_A the mean aggregation number of the formed micelles is much lower than with purely aqueous solutions.

For $\varphi_A > 10\%$ the CMC was determined by a photometric method. This method has been already presented in [15]. It is based on the influence of the micelle formation on the solubility of very non-polar compounds in aqueous solutions. This method was not applicable with solutions of $\varphi_A > 20\%$. With high φ_A the solubility of anthracene in solutions without a micellar phase is too high to allow precise evaluation of the CMC from the measurement curves.

The CMC of SDS at 30°C for various φ_A at constant ionic strength of the buffer are shown in Table 1. The value determined with the photometric method is slightly lower than the value determined with the conductometric method. This difference might be due to the presence of anthracene in solutions examined with the photometric method. From the data in Table 1 it can be concluded that the influence of φ_A on the CMC is very low. The variation of φ_A from 0–20% results in an increase of CMC from 4.9-to 6.1 mM. These results suggest that the phase ratio V_s/V_m is influenced by φ_A to a small extent in the range examined.

3.2. Correlation studies

In MEKC the resolution of two closely adjacent peaks is dependent on the efficiency of the chromatographic system, the selectivity factor for the two solutes, the mean retention factor and the ratio of the migration time of the mobile phase to the migration time of the micelles according to Eq. (1). The retention factor of the first and the second solute, contained in the original equation, was replaced by the mean retention factor. Eq. (1) can be directly employed for calculating the resolution of any peak pair for any electrolyte composition, if the dependence of the magnitudes in Eq. (1) (efficiency,
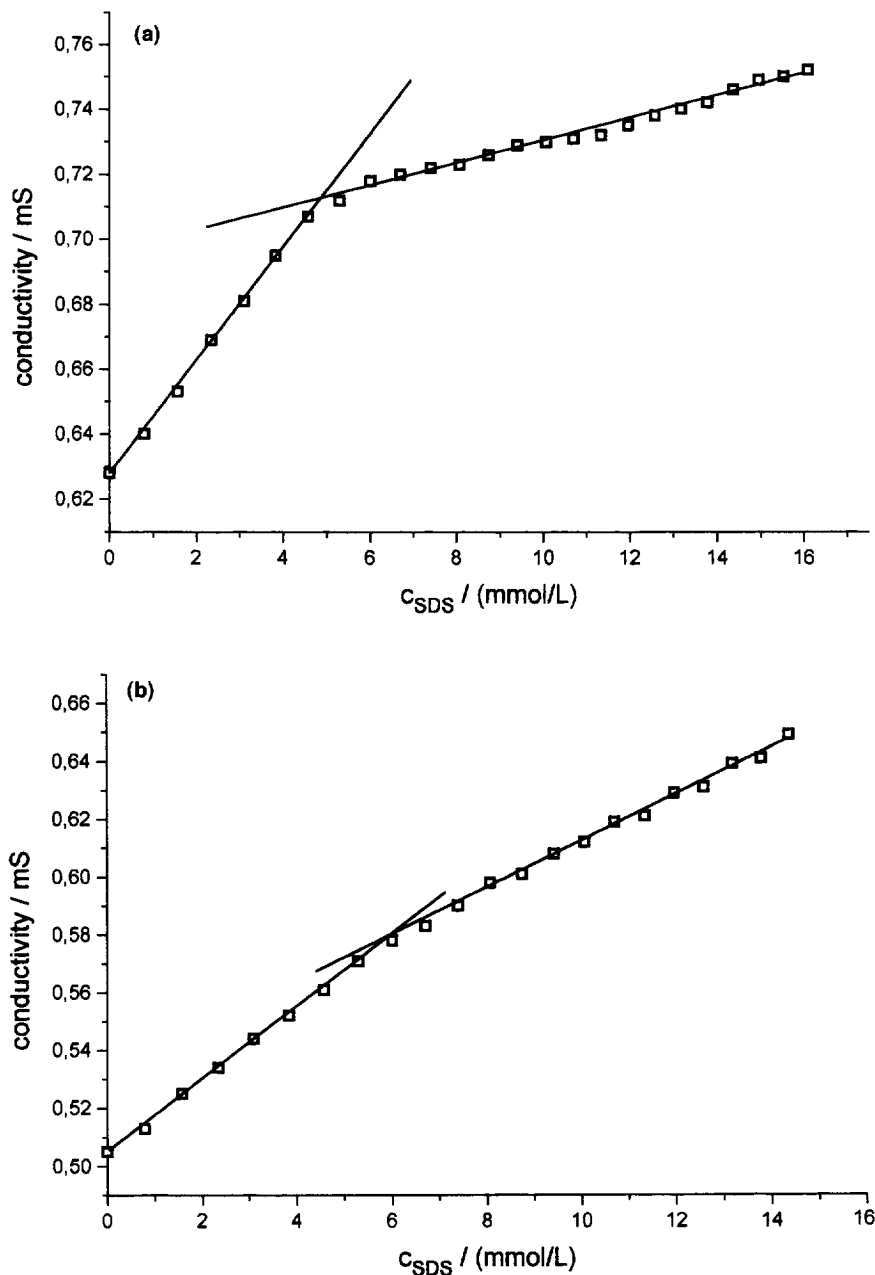
Fig. 1. Conductometric determination of the critical micellar concentration (CMC), conductivity vs. surfactant concentration. (a) $\varphi_A = 0\%$ (b) $\varphi_A = 10\%$ (measurement conditions: $c_{Na_2HPO_4} = 6.25$ mM, $c_{Na_2B_4O_7} = 1.25$ mM, $T = 30°C$)

selectivity factor, retention factor and t_0/t_M) on the electrolyte composition is known.

In the following calculations, the efficiency of the chromatographic system is considered to be independent of the electrolyte composition. All other quan-

tities: k, α, t_0 and t_M are dependent on the electrolyte composition. For convenience, in place of the volume fraction φ_A the volume concentration of acetonitrile σ_A was employed as magnitude to quantify the content of acetonitrile in the separation electrolyte.

Table 1
The dependence of the critical micelle concentration (CMC) of SDS on the volume fraction of acetonitrile φ_A

φ_A (%)	CMC (mM)
0	4.89[a]
5	5.17[a]
10	6.03[a]
10	5.09[b]
15	5.78[b]
20	6.09[b]

[a] Conductometric method
[b] Photometric method $c_{Na_2HPO_4}=6.25$ mM, $c(Na_2B_4O_7)=1.25$ mM, $T=30°C$.

In the first experimental step, the function $y = f(c_{SDS}, \sigma_A)$ was determined that best describes the dependence of k, t_0 and t_M on the surfactant concentration (c_{SDS}) and the modifier concentration (σ_A). Caffeine, p-nitrotoluene and naphthalene were used as test solutes. The surfactant concentration in the separation electrolyte was varied from 20–100 mM. The acetonitrile concentration varied from 0–15%. The concentration of disodium tetraborate and boric acid in the separation electrolyte was kept constant at 10 mM in all measurements.
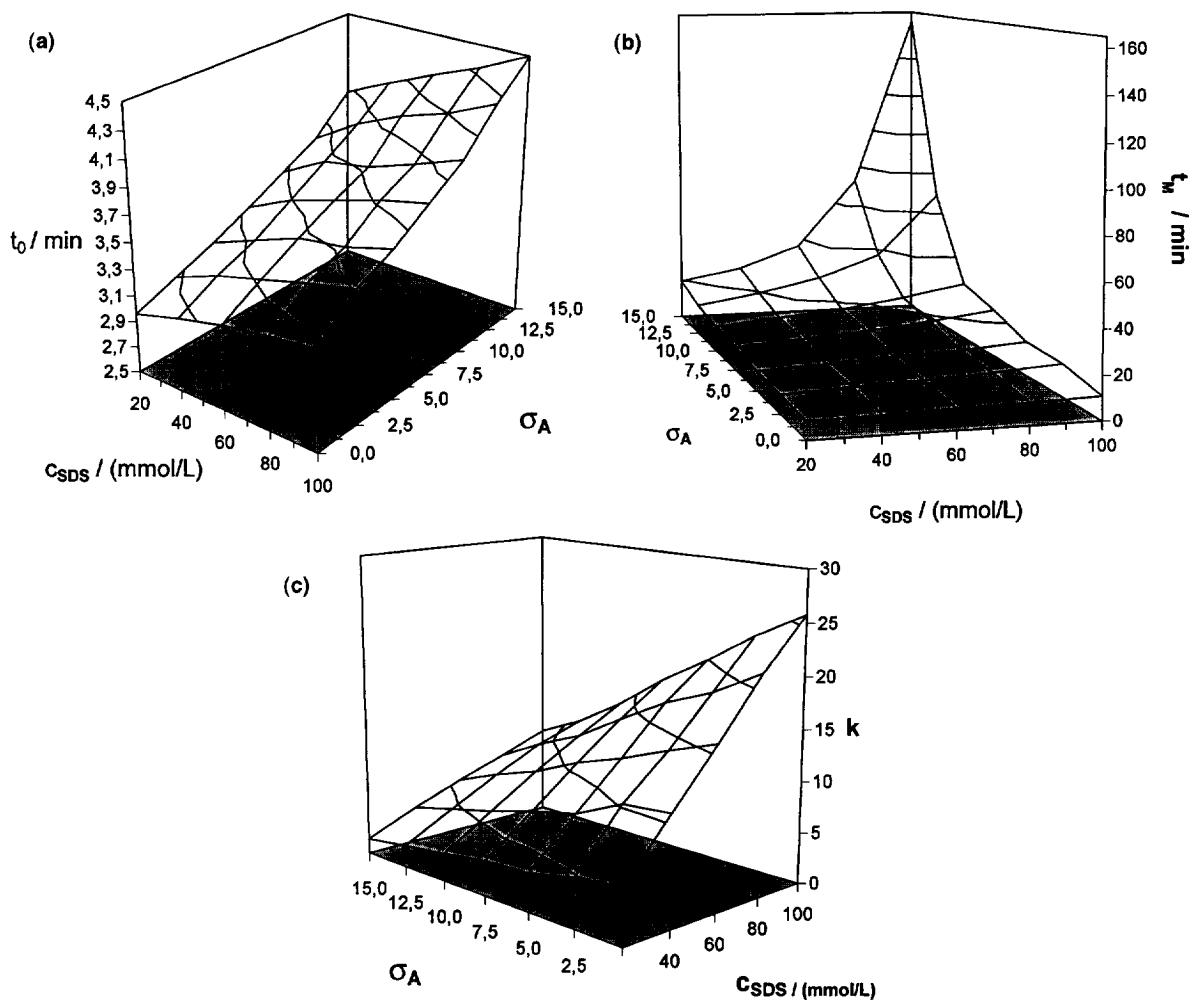
In Fig. 2a–c t_0, t_M and the retention factor of

Fig. 2. Dependence of (a) the hold-up time t_0 (b) the migration time of the micelles t_M and (c) the retention factor k of naphthalene on the surfactant and modifier concentration. Experimental conditions: Capillary, 565 (500) mm×75 μm I.D.; buffer, $c_{Na_2B_4O_7}=10$ mM, $c_{H_3BO_3}=10$ mM; voltage, 25 kV; temperature, 25°C; injection, pressure, 2 s; detection, photometric, 254 nm.

naphthalene are plotted against c_{SDS} and σ_A. For t_0 the resulting area can be described by the function:

$$t_0 = a + bc_{SDS} + d\sigma_A + ec_{SDS}\sigma_A$$

$$(a,b,d,e = constants);\ r^2 = 0.988 \tag{2}$$

It is interesting to note that with high c_{SDS} the apparent migration velocity of the micelles, v_M, is strongly dependent on the content of acetonitrile. This dependence is only given for $c_{SDS} \geq 70$ mM and has not been reported by other authors, who investigated v_M at low c_{SDS} (20–50 mM) [16–20]. Drastic changes in the electrophoretic mobility of the micelles can be explained with alterations of the micelle structure due to an influence of the organic modifier and the surfactant concentration on the balance of forces governing the formation of micelles [20].

Best fit was found for t_M with the function:

$$\log t_M = a' + b'c_{SDS} + d'\sigma_A^2 + e'c_{SDS}^4\sigma_A^5$$

$$(a',b',d',e' = constants);\ r^2 = 0.984 \tag{3}$$

If v_M is increased, while the electroosmotic velocity is affected to a lesser extent, the ratio t_0/t_M, a measure of the elution window, is decreased. According to Eq. (1) the resolution is improved with lowered t_0/t_M. In Fig. 3 t_0/t_M is plotted against c_{SDS} and σ_A. At $c_{SDS} = 80$ mM and $\sigma_A = 0.15$ a nearly infinite elution range is approached ($t_0/t_M = 0.06$)
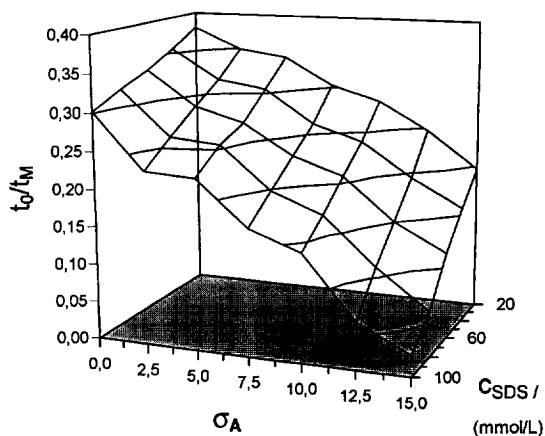

Fig. 3. Dependence of the ratio of hold-up time t_0 and migration time of the micelles t_M on the surfactant and modifier concentration. Experimental conditions: see Fig. 2.

with a migration time of the micelles of 72.3 min. The usefulness of an infinite elution range in MEKC (and a quasi-'stationary' pseudostationary phase) has recently been highlighted by Ahuja et al. [23]. An infinite elution range enables difficult separations at the expense of long run times.

Best fit was found for the retention factors with the function:

$$k = a'' + b''c_{SDS} + d''\sigma_A + e''c_{SDS}\sigma_A$$

$$(a'',b'',d'',e'' = constants);$$

$$r^2(p\text{-nitrotoluene}) = 0.995; \tag{4}$$

$$r^2(\text{naphthalene}) = 0.998$$

The constants of Eqs. (2)–(4) can be calculated from four test runs with varied surfactant and acetonitrile concentrations with a linear equation system given.

3.3. Employed algorithm

The derived equations (Eqs. (2)–(4)) were used to calculate the data base (a set of constants) for subsequent predictions of optimum conditions for separations in MEKC. Optimum is defined here as the condition that allows a separation to be performed in the shortest time, while the resolution of the worst-separated peak pair exceeds a threshold value.

In the first step, the hold-up time, the migration time of the micelles and the retention times of the solutes of interest are entered for four different electrolyte compositions (varied c_{SDS} and σ_A). The selected values of c_{SDS} and σ_A should form a square, covering the area of interest. The user of the computer program also selects the minimum and the maximum values for c_{SDS} and σ_A, used for resolution optimization. Selected also is the minimum resolution that has to be reached for the worst resolved peak pair and the plate number that is approximated to be constant for all electrolyte compositions. The user can also fix the step-width of the underlying algorithm (Δc_{SDS}, $\Delta \sigma_A$). Optionally, the user can label some solutes as 'impurities'. The resolution of labeled solutes from each other is not taken into account in the optimization procedure.

In a second step, the retention factors for the four electrolyte compositions are calculated for all sol-

utes. Consecutively, the constants of Eqs. (2)–(4) are calculated for t_0, t_M and the retention factors of all solutes of interest. With these constants, the retention times, t_R, for all solutes are accessible for any value of c_{SDS} and σ_A according to Eq. (5):

$$t_R = (t_0 k + t_0)/(1 + k(t_0/t_M)) \qquad (5)$$

The bandwidth, w, (4σ) is accessible for any t_R employing Eq. (6):

$$w = (4/\sqrt{N})t_R \qquad (6)$$

With these magnitudes the resolution, R_s, for every peak can be calculated according to:

$$R_s = (t_{R,2} - t_{R,1})/[(w_2 + w_1)/2] \qquad (7)$$

where: $t_{R,2}$ = retention time for the second solute; $t_{R,1}$ = retention time for the first solute; w_2 = peak width of the second peak; w_1 = peak width of the first peak.

If the elution order is dependent on the electrolyte composition, the resolutions for each possible peak pair have to be calculated. Optionally, for each electrolyte composition taken into account in the optimization procedure (depending on the minimum and the maximum values for c_{SDS}, σ_A, Δc_{SDS}, and $\Delta \sigma_A$) the software generates a list of migration times for all solutes. Additionally, the resolution of the worst-separated peak pair is given for each electrolyte composition. These optional features of CABRO II are control devices.

In the third step, the resolutions obtained for various electrolyte compositions are compared to each other. The program suggests an optimum electrolyte composition, where the resolution of the worst-separated peak pair exceeds a user-defined threshold value and the retention time of the last eluted solute is minimum. The retention time of the last eluting solute is approximately equivalent to the time of the chromatographic run. Thus, the program performs an adjustment of the electrolyte composition for a minimum time of analysis.

Optionally, the electrolyte composition can be calculated, where the resolution of the worst-separated peak pair is the highest in the selected range regardless of analysis time. The maximum attainable value of R_{min} is referred to as R_{max}. R_{max} corresponds to the best resolution of the worst-separated peak pair that can be obtained with SDS as surfactant and acetonitrile as modifier in the selected concentration range. If in the selected range the resolution of the worst-separated peak pair (R_{min}) does not exceed the user-defined threshold value, the electrolyte composition corresponding to R_{max} is suggested by the program as the optimum electrolyte composition.

In the final step, the employed program generates a list of retention times predicted for the optimized electrolyte composition and a simulated chromatogram.

3.4. The separation of biogenic amines

It is known that biogenic amines are present in a variety of foods including cheese, fish and meat and in beverages such as wine and beer [24]. Because of the potential toxicity of these compounds it is desirable to have a simple and rapid method of quantitation of biogenic amines in trace amounts (mg l^{-1}) in foods and beverages. The methods most commonly used in the determination of biogenic amines are thin layer chromatography [25–27], gas chromatography [28–30] and high-performance liquid chromatography (HPLC) [31–37]. At the present time HPLC is the preferred method. Recently, Nouadje et al. [38,39] have presented a method for the determination of pressor amines and some principal amino acids in wine and amines in dairy products with a combination of MEKC and laser-induced fluorescence detection (LIFD). The amines and amino acids were derivatized with fluorescein isothiocyanate. The chromatographic runs take less than 25 min.

In routine procedures, the amines are derivatized prior to sample clean-up, pre-concentration and determination. The most commonly used amine derivatizing agents suitable for fluorescence and photometric detection after chromatographic separation are o-phthaldialdehyde (OPA) and dansyl chloride. OPA has the disadvantage that it reacts only with primary amines and its derivatives are relatively unstable. Therefore, dansyl chloride has been considered to be the agent to be preferred.

Derivatives with dansyl chloride are non-polar. They are easily extractable from aqueous solutions. Preliminary studies in our laboratory have shown,

that derivatized amines cannot be separated by MEKC, if urea is taken as modifier. Also with high urea content in the separation electrolyte the retention factors of the solutes are not in the optimum range.

Acetonitrile as modifier in MEKC reduces the retention factors to a higher extent than urea. Therefore, CABRO II has been employed for the optimization of the separation of 14 derivatized biogenic amines by MEKC. The buffer compositions of the four test runs are as follows: Run 1, $c_{SDS} = 20$ mM, $\sigma_A = 0.15$; Run 2, $c_{SDS} = 80$ mM, $\sigma_A = 0.15$; Run 3, $c_{SDS} = 40$ mM, $\sigma_A = 0.075$; Run 4, $c_{SDS} = 100$ mM, $\sigma_A = 0.10$. The concentration of $Na_2B_4O_7$ and of H_3BO_3 was in all cases 10 mM in the prepared

separation buffer. The chromatograms obtained under the conditions of the test runs are shown in Fig. 4a to d. In Run 3 and 4 (Fig. 4c to d) the solutes are insufficiently separated. The run time of Run 2 exceeds 50 min. As discussed in the previous section under the conditions of this run a nearly infinite elution range is obtained. Under conditions of Run 1 a sufficient separation of the solutes is achieved at a moderately short run time of 20 min.

CABRO II generates a list of resolutions of the worst separated peak pair (R_{min}) for any possible buffer composition within the selected parameter range, given a user defined step-width. The parameter space was set at: $c_{SDS} = 20$–100 mM, $\sigma_A = 0.01$–0.15. It corresponds to the useful experimental range taking
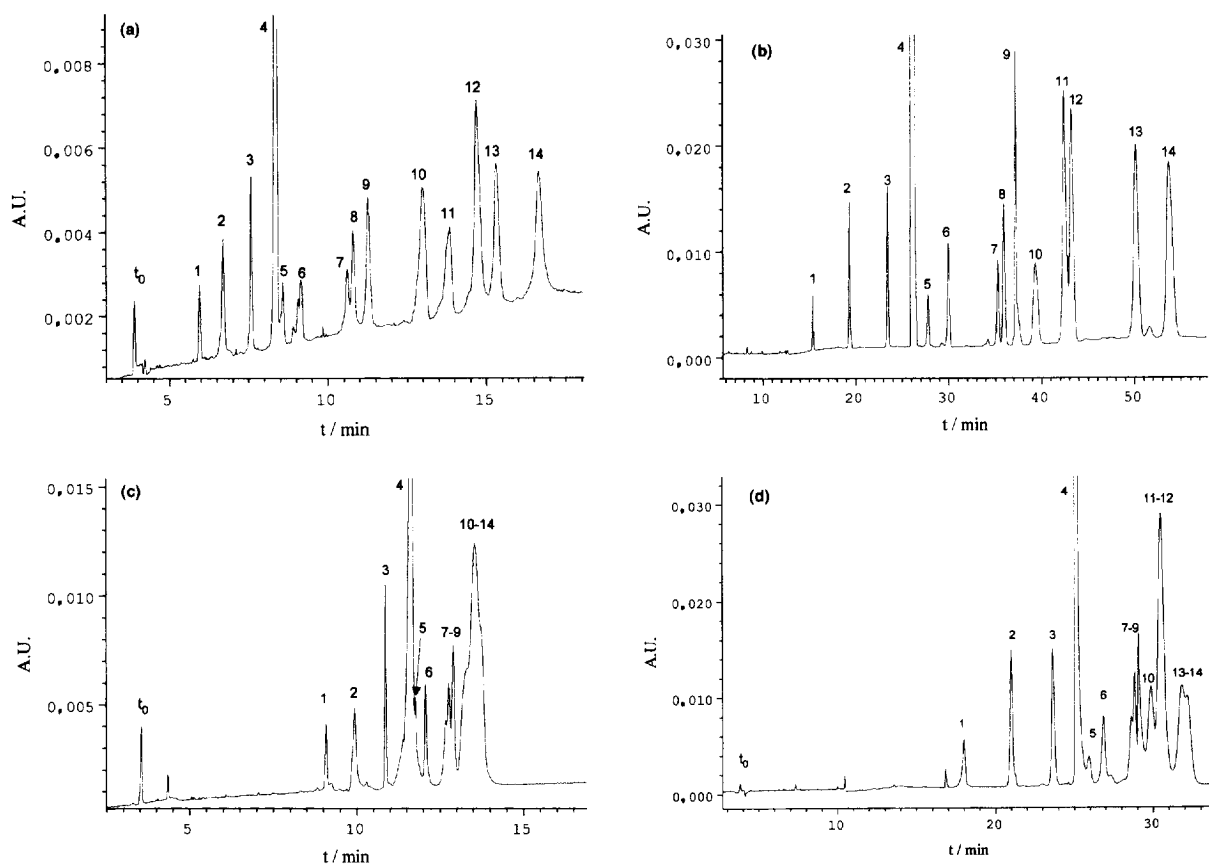

Fig. 4. Separation of dansylated biogenic amines with various SDS and acetonitrile concentrations. Buffer composition: $c_{Na_2HPO_4} = 10$ mM, $c_{H_3BO_3} = 10$ mM; (a) $\sigma_A = 15\%$, $c_{SDS} = 20$ mM; (b) $\sigma_A = 15\%$, $c_{SDS} = 80$ mM; (c) $\sigma_A = 7.5\%$, $c_{SDS} = 40$ mM; (d) $\sigma_A = 10\%$, $c_{SDS} = 100$ mM. Solutes: 1 = 2-aminoethanol, 2 = methylamine, 3 = ethylamine, 4 = morpholine, 5 = isopropylamine, 6 = propylamine, 7 = diethylamine, 8 = isobutylamine, 9 = 1-butylamine, 10 = 3-methylbutylamine, 11 = 1,4-diaminobutane, 12 = 1,5-diaminopentane, 13 = hexylamine, 14 = heptylamine. Experimental conditions: see Fig. 2.

the CMC and the solubility of SDS into consideration.

R_{min} is the primary optimization criterion. A contour plot of R_{min} within the parameter space investigated is given in Fig. 5. Maximum values of the primary resolution criterion R_{min} are reached at the borders of the selected parameter range. Two local maxima are at $c_{SDS} = 20$ mM, $\sigma_A = 0.15$ and $c_{SDS} = 80$ mM, $\sigma_A = 0.15$. If R_{min} exceeds a user-defined threshold value ($R_{min,t}$), the retention time of the last eluted solute is the secondary optimization criterion within the parameter range fulfilling the threshold criterion.

With the plate number, N, set at 100 000 and $R_{min,t} = 1.4$, CABRO II selects the buffer composition employed in Run 1 ($c_{SDS} = 20$ mM, $\sigma_A = 0.15$) as optimum. With lowered threshold criterion ($R_{min,t} = 1.2$ and $R_{min,t} = 1.0$) buffer compositions with lower σ_A are suggested by the program: $c_{SDS} = 30$ mM, $\sigma_A = 0.13$, $R_{min} = 1.20$ and $c_{SDS} = 25$ mM, $\sigma_A = 0.13$, $R_{min} = 1.06$, respectively. With buffer compositions with lower σ_A the run time (migration time of the last eluted solute) is slightly reduced.

The ability of CABRO II to predict solute migration times was tested with following buffer compositions: (a) $c_{SDS} = 50$ mM, $\sigma_A = 0.05$ and (b) $c_{SDS} = 20$ mM, $\sigma_A = 0.10$. Predicted and measured migration times are listed in Table 2. In Table 2 also the migration time differences 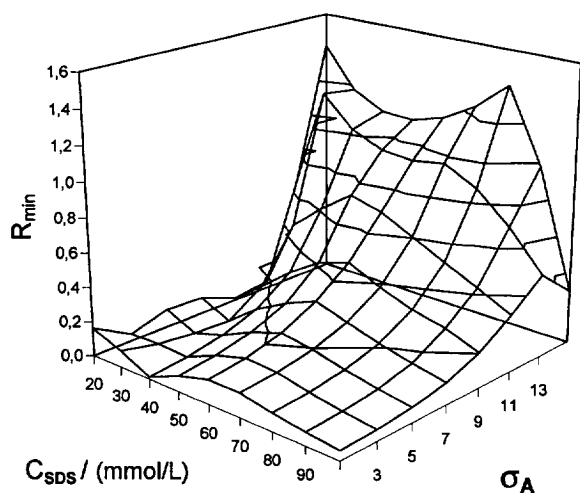for two adjacent peaks are given. The predicted migration time differences correspond well with the measured migration time differences. In (a) the predicted migration times are slightly underestimated with a constant value of 2 min. In (b) the predicted migration times are slightly overestimated with 1.0–1.5 min. This deviation can be explained by the high standard deviation of the regression planes for t_0 and t_M.

3.5. Reproducibility and efficiency

With $\sigma_A \geq 0.10$ we observed an intolerable instability of the EOF, if the capillary is rinsed routinely between two runs only with the separation electrolyte. This instability was overcome by rinsing the capillary between two runs successively with concentrated nitric acid (5 min), water (2 min), aqueous solution of NaOH ($c = 0.1$ M; 2 min), water (2 min) and separation electrolyte (3 min).

With high acetonitrile content and low surfactant concentration in the separation electrolyte, the solute zones exhibit a characteristic fronting (see Fig. 4a), also observed by Brüggemann and Freitag [19]. This fronting results in a drastic reduction of the efficiency. The fronting obtained with high acetonitrile content in the separation electrolyte is not yet discussed in the literature according to our knowledge. Our measurements of the electric conductivity suggest that at higher φ_A the mean aggregation number of the formed micelles is much lower than in purely aqueous solutions. Inhomogenities of the micellar structure (microheterogenity resulting from polydispersity of the micelle size [40]) might be responsible for the fronting observed. It should be noted that the solutes exhibit no electrophoretic mobility.

The efficiency is also distinctively reduced, if the derivatizing agent dansyl chloride is not completely converted into acid or amide. Obviously dansyl chloride in excess is able to react with the inner surface of the fused-silica capillary, forming a monolayer that interacts strongly with the non-polar solutes. Varying migration times and intolerable band broadening are the characteristics of a modified inner surface of the capillary.

With the conditions of derivatization described in Section 2.3, however, no such difficulties have been

Fig. 5. Contour plot of the optimization criterion R_{min} in dependence on the surfactant and modifier concentration.

Table 2
Comparison of predicted and measured migration times

Component	Predicted retention time (min)	Measured retention time (min)	Predicted time difference between adjacent peaks (min)	Measured difference between adjacent peaks (min)
(a) Buffer composition: $\sigma_A = 0.05$, $c_{SDS} = 50$ mM, $c_{Na_2B_4O_7} = 10$ mM, $c_{H_3BO_3} = 10$ mM				
2-Aminoethanol	9.32	10.99	0.74	0.97
Methylamine	10.06	11.96	0.72	0.84
Ethylamine	10.78	12.80	0.51	0.50
Morpholine	11.29	13.30	0.12	0.10
Isopropylamine	11.41	13.40	0.22	0.30
Propylamine	11.63	13.70	0.39	0.60
Diethylamine	12.02	14.30	0.06	<0.1
Isobutylamine	12.08	14.30	0.07	<0.1
1-Butylamine	12.15	14.30	0.24	0.40
3-Methylbutylamine	12.39	14.70	0.02	<0.1
1,4-Diaminobutane	12.41	14.70	0.06	<0.1
1,5-Diaminopentane	12.47	14.70	0.09	<0.1
Hexylamine	12.56	14.70	0.10	<0.1
Heptylamine	12.66	14.70	–	–
(b) Buffer composition: $\sigma_A = 0.10$, $c_{SDS} = 20$ mM, $c_{Na_2B_4O_7} = 10$ mM, $c_{H_3BO_3} = 10$ mM				
2-Aminoethanol	7.60	6.63	0.57	0.82
Methylamine	8.17	7.45	1.07	0.94
Ethylamine	9.24	8.39	1.32	0.69
Morpholine	10.56	9.08	0.01	0.08
Isopropylamine	10.57	9.16	0.50	0.44
Propylamine	11.07	9.60	1.00	1.03
Diethylamine	12.07	10.63	0.19	0.14
Isobutylamine	12.26	10.77	0.30	0.21
1-Butylamine	12.56	10.98	0.77	0.42
3-Methylbutylamine	13.33	11.40	0.01	0.10
1,4-Diaminobutane	13.34	11.50	0.21	0.30
1,5-Diaminopentane	13.55	12.20	0.03	0.10
Hexylamine	13.58	12.30	0.52	0.30
Heptylamine	14.10	12.60	–	–

Measurement conditions see Fig. 2.

observed. Band broadening has been observed only if lower temperature or shorter reaction time have been employed for the derivatization reaction.

σ_A-parameter space on the basis of only four initial experiments.

4. Conclusions

The composition of the separation electrolyte (concerning the SDS and acetonitrile concentration) can be rapidly optimized for a complex mixture of solutes by the developed computer-aided procedure. With the program presented it is possible to predict migration times of neutral solutes for a given c_{SDS} –

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