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

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Abstract

A computer-aided method is presented that permits the simultaneous optimization of the concentration of an anionic surfactant (sodium dodecyl sulfate) and the modifier urea in the separation buffer in micellar electrokinetic chromatography (MEKC) after only three test runs. The underlying algorithm takes into account that resolution in MEKC is dependent on selectivity, retention factor and the ratio of the hold-up time to the migration time of the micelles (t_0/t_M). All these factors are influenced by the buffer composition. The optimization method presented was applied to the separation of nitroaromatic compounds, urea pesticides and aliphatic amines derivatized with *p*-nitrobenzoic acid.

1. Introduction

Micellar electrokinetic chromatography (MEKC), first introduced by Terabe and coworkers [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). Since its introduction in 1985, MEKC has already found various applications, especially in the analysis of nitroaromatic compounds [3,4] and in pharmaceutical analysis [5,8].

In spite of the growing importance of MEKC, there are only a few examples of computer-assisted systematic resolution optimization for this separation technique. Vindevogel and Sandra [9] used a Plackett–Burman statistical design to optimize the resolution of testosterone esters. The method required eight experiments for the optimization of five parameters. Yik and Li [10] used a three-dimensional overlapping resolution mapping scheme to optimize the resolution of dinitrophenyl-derivatized amino acids. The method required eleven preplanned experiments and predicted the optimum buffer pH, surfactant [sodium dodecyl sulfate (SDS)] concentration and modifier (tetrabutylammonium salt) concentration.

In the approach of Yik and Li [10], a theoretical model to describe the migration behaviour is not required. The same holds for the method of Vindevogel and Sandra [9]. Smith and Khaledi [11] tested another approach: they predicted the migration behavior of a homologous series of phenols over a two-dimensional pH–micelle concentration space. The model describes mobility in terms of the acid dissociation constant, the partition coefficient, the pH of the buffer and the

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micelle concentration. Eighteen experiments were necessary to obtain the data set required for weighted non-linear regression in order to evaluate the physical and chemical constants of each solute. These constants form the data basis for subsequent predictions.

Quang et al. [12] 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. The concentration of additives, however, is not taken into account in their optimization approach.

In a recent paper [13], we investigated whether at a given pH the buffer composition concerning the surfactant (SDS) concentration and the modifier (urea or glucose, respectively) concentration can be optimized rapidly by employing the basic equation for resolution (R_s) developed by Terabe and co-workers [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, α = selectivity factor, t_0 = migration time of the mobile phase and t_M = migration time of the micelles. We predicted the optimum surfactant or modifier concentration, keeping constant all other parameters, on the basis of only two test runs.

Terabe et al. [2] had already shown that the function $f(k) = [k/(1+k)]\ (1 - t_0/t_M)/[1 + (k \cdot t_0/t_M)]$ passes through a maximum if $t_0 \neq t_M$. Foley [14] 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. He recommended tuning the phase ratio (volume of pseudo-stationary phase/volume of mobile phase) by varying the surfactant concentration in order to adjust the retention factors of the solutes to the derived optimum condition. In practice, however, with varying surfactant concentrations t_0/t_M is also strongly influenced [2,12,15]. This influence cannot be neglected in optimization algorithms.

Little and Foley [16] have shown in an experimental study that ignoring the influence of the surfactant concentration on t_0/t_M renders incorrect predictions of the optimum buffer composition. They therefore suggested keeping the ratio t_0/t_M constant by adding a non-ionic surfactant to the separation buffer in order to simplify the optimization procedure. It must be stressed that this approach will only be successful if the solutes differ sufficiently in their retention factors. Generally, the ratio t_0/t_M should be minimized (while keeping t_0 constant) as much as possible to enhance the resolution according to Eq. 1. In our approach [13], the surfactant concentration can be rapidly optimized, although t_0/t_M is largely dependent on the concentration of the surfactant examined (SDS).

Urea is reported [17] to be a versatile modifying additive to the mobile phase that improves the ratio t_0/t_M to a great extent without reducing strongly the electroosmotic velocity, v_{eo}. The addition of urea to the mobile phase also decreases the retention factors of the solutes, thus making it possible to adjust them to the optimum condition derived by Foley [14]. The urea and the surfactant concentrations are two important factors in controlling migration and resolution of neutral solutes. Owing to the interactive nature of these two factors, the simultaneous optimization of the urea and surfactant concentrations in the running buffer employed in MEKC is therefore desirable for many applications.

Alternatively, a reduction in the electroosmotic velocity, either by coating the inner wall of the capillary or by reducing the buffer pH, can be used to decrease the ratio t_0/t_M [15]. However, these methods increase the analysis time. Other organic modifiers have been used besides urea: acetonitrile [18], glucose [19], 2-propanol [20] and methanol [18]. These modifiers have the disadvantage that they reduce considerably the electroosmotic velocity resulting from their influence on viscosity and dielectric constant of the separation buffer [13,18–21]. Up to the present, only urea has been reported as a modifier that improves the elution range without substantially reducing the electroosmotic velocity [13,18].

Another possibility for improving the elution range without influencing the electroosmotic velocity is offered by reducing the chain length of the anionic buffer employed [15]. Modifying the chain length of the surfactant, however, can

have an undesirable effect on the critical micellar concentration (CMC) of the surfactant. With anionic surfactants, the CMC is reported to increase with shorter chain lengths [22]. SDS has been reported to have a low CMC under the conditions normally employed in MEKC [13]. The CMC of SDS is virtually unaltered by the addition of urea to the separation buffer.

If the separated solutes have an electrophoretic mobility, not only the apparent retention factors and t_0/t_M are influenced by the buffer composition but also the apparent selectivity factors, α. The apparent selectivity factor for two solutes is the ratio of their apparent retention factors, ignoring electrophoretic effects.

In this paper, a computer-aided method based on a computer program called computer-assisted bivariate resolution optimization (CABRO) is presented that permits the prediction of the optimum buffer composition concerning the concentration of sodium dodecyl sulfate (SDS) and urea on the basis of only three test runs. Changes in the apparent selectivities, the retention factors and t_0/t_M are taken into consideration in the algorithm employed.

2. Experimental

2.1. Reagents

Chemicals used for separation studies were purchased from various suppliers. Sodium tetraborate, boric acid and urea (Merck, Darmstadt, Germany) and sodium dodecyl sulfate (Roth, Karlsruhe, Germany) used for the preparation of the separation buffers were of analytical-reagent grade. The urea pesticide standards were purchased from Riedel-de Haën (Seelze, Germany). Water was doubly distilled.

2.2. Derivatization of amines

The amines were dissolved in dry acetone. 4-nitrobenzoyl chloride was added in excess and the solution was kept at 50°C for 20 min. The solution was then made alkaline by adding an equal volume of aqueous disodium tetraborate (50 mmol/l). The solution was shaken and then left for 20 min at 70°C in order to evaporate the acetone and to convert the excess of 4-nitrobenzoyl chloride into 4-nitrobenzoic acid.

2.3. Chromatographic measurements

All chromatographic measurements were carried out with a Beckman (Fullerton, CA, USA) 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 1–2 s. Detection was performed at 254 nm. All separations were carried out at 25 kV. Data were recorded with 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 capillary rinsing procedures employed have already been presented in detail [23]. The elution time of the mobile phase, t_0, and the elution time of the micellar phase, t_M, were determined using formamide or thiourea and quinine hydrochloride, respectively, as markers. Peak identities were confirmed by spiking.

Studies with various concentrations of SDS and urea were performed either with a buffer containing 10 mmol/l H_3BO_3 and 10 mmol/l $Na_2B_4O_7$ or with a buffer containing 30 mmol/l $Na_2B_4O_7$.

2.4. Optimization software

All programs used for separation studies were written in Pascal employing Turbo Pascal 6.0.

3. Results and discussion

3.1. 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 that of the micelles according to Eq. 1. The retention factor of the first and the second solutes, contained in the original equation, was replaced by the mean retention factor.

In the following calculations, the efficiency of the chromatographic system is considered to be independent of the buffer composition. All other quantities, k, t_0 and t_M, are dependent on the buffer composition; α is partially dependent on the surfactant and the modifier concentration [13].

In the first experimental step, the function $y = f(c_{SDS}, c_{urea})$ was determined that best describes the dependence of k, t_0 and t_M on the surfactant concentration (c_{SDS}) and the modifier concentration (c_{urea}). Caffeine, *p*-nitrotoluene, 4-methyl-3-nitroaniline, 4-methyl-2-nitroaniline, 2-methyl-3-nitroaniline and naphthalene were used as test solutes. The surfactant concentration in the separation buffer was varied from 20 to 100 mmol/l in 20 mmol/l steps. The urea concentration was varied from 0.0 to 4.0 mol/l in 0.5 mol/l steps. The concentration of disodium tetraborate in the separation buffer was kept constant at 30 mmol/l in all measurements.

In Fig. 1a–e, t_0, t_M and the retention factors of caffeine, *p*-nitrotoluene and naphthalene are plotted against c_{SDS} and c_{urea}. It can be seen that for t_0 the resulting area can be approximated by a plane, described by the function

$$t_0 = f(c_{SDS}, c_{urea}) = a + bc_{SDS} + dc_{urea} \quad (2)$$

where a, b and d are constants. The best fit was found for t_M with the function

$$\ln t_M = f(c_{SDS}, c_{urea}) = a' + b' \ln(c_{SDS}) + d'c_{urea} \quad (3)$$

where a', b' and d' are constants.

In Fig. 1f, $\ln t_M$ is plotted against $\ln c_{SDS}$ and c_{urea}. Whereas in Fig. 1b the resulting area is strongly vaulted, in Fig. 1f the area of the functional values can be approximated by a plane.

Terabe et al. [17] have reported that the logarithm of the retention factor for a solute decreases linearly with increasing concentration of urea, if c_{SDS} is kept constant:

$$\ln k = \text{constant} + \text{constant}' \cdot c_{urea} \quad (4)$$

In early studies [2], it was shown that k is linearly dependent on c_{SDS} above the CMC. It was found that the CMC of SDS is between 1 and 4 mmol/l in the buffers usually employed in MEKC [13]. If the small y-intercept of the function $k = f(c_{SDS})$ is neglected, the two functions $k = f(c_{SDS})$ and $k = f(c_{urea})$ can be combined by the following equation:

$$\ln k = f(c_{SDS}, c_{urea}) = a'' + b'' \ln c_{SDS} + d''c_{urea} \quad (5)$$

where a'', b'' and d'' are constants. The area resulting from the function $k = f(c_{SDS}, c_{urea})$ can also be approximated by a plane (Fig. 1c–e). The regression coefficients of regressions keeping one of the parameters (c_{SDS} or c_{urea}) constant are significantly improved with Eq. 5 compared with the regression coefficients of regressions keeping one of the parameters (c_{SDS} or c_{urea}) constant and approximating the function $k = f(c_{SDS}, c_{urea})$ by Eq. 8.

The constants of Eqs. 2, 3 and 5 can be calculated from just three test runs with various surfactant and urea concentrations assuming a linear equation system.

3.2. Algorithm employed

The empirical equations (Eqs. 2, 3 and 5) 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 three different buffer compositions (various c_{SDS} and c_{urea}). The selected values of c_{SDS} and c_{urea} should form a triangle, covering the area of interest. The user of the computer program also

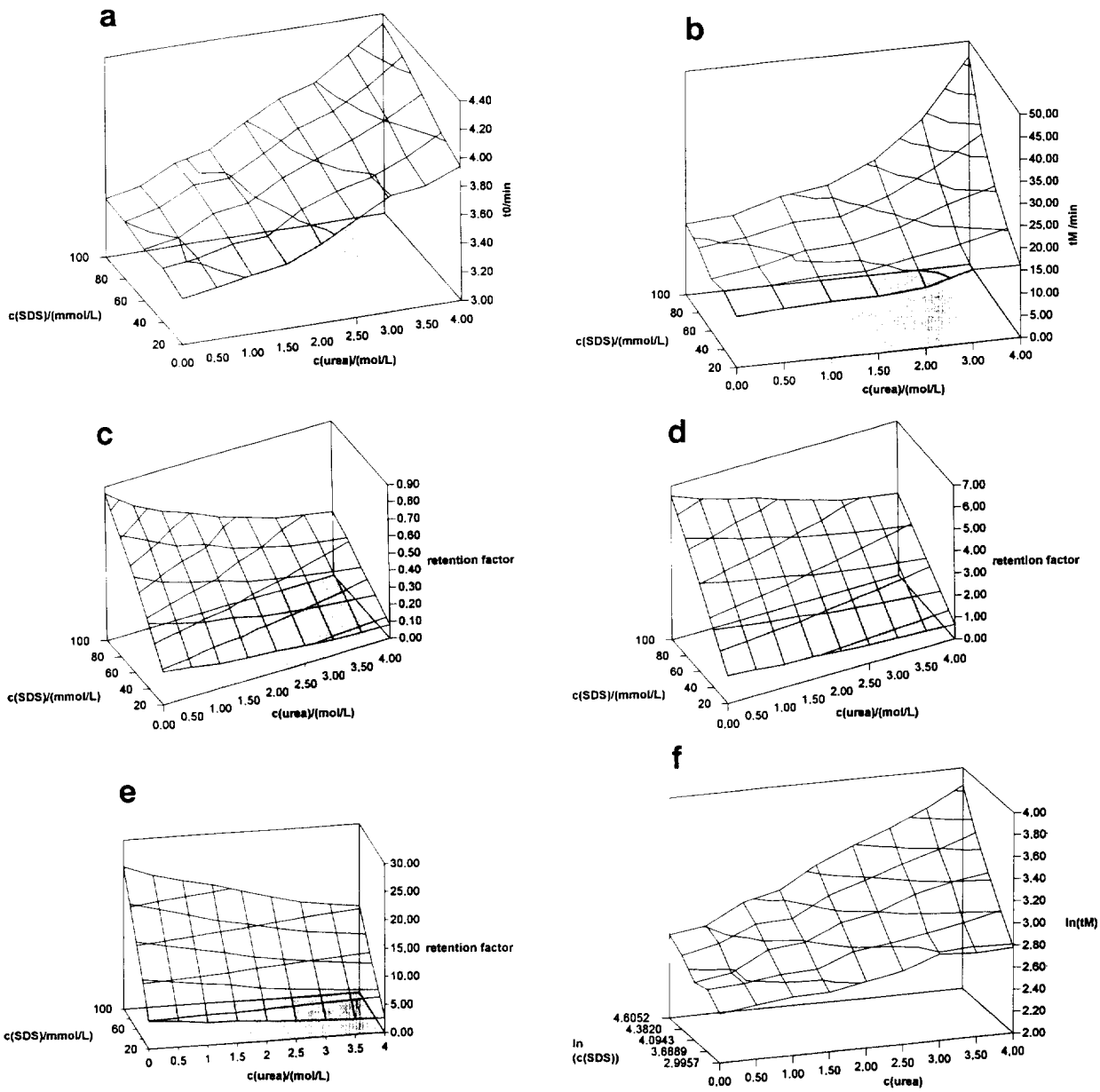

Fig. 1. Dependence of (a) the hold-up time (marker: formamide) on c_{SDS} and c_{urea}, (b) the migration time of the micelles (marker: quinine hydrochloride) on c_{SDS} and c_{urea}, (c–e) the retention factor of caffeine (c), *p*-nitrotoluene (d) and naphthalene (e) on c_{SDS} and c_{urea}, (f) the logarithm of the migration time of the micelles on ln c_{SDS} and c_{urea}. Capillary, 565 (500) mm × 75 μm I.D.; buffer, $c(Na_2B_4O_7)$ = 30 mmol/l; voltage, 25 kV; temperature, 25 °C; injection, pressure, 2.0 s; detection, photometric, 254 nm.

selects the minimum and the maximum values for c_{SDS} and c_{urea}, used for resolution optimization. Selected is also 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 buffer compositions. The user can also fix the step width of the underlying algorithm (Δc_{SDS}, Δc_{urea}).

In a second step, the retention factors for three buffer compositions are calculated for all

solutes. Consecutively, the constants of Eqs. 2, 3 and 5 are calculated for t_0, t_M and the retention factors of all solutes of interest. With these constants, the resolution for two adjacent peaks is accessible for any value of c_{SDS} and c_{urea} according to Eq. 1. The variable k is determined for each peak pair from the retention factors of solutes eluting in succession:

$$k = (k_1 + k_2)/2 \quad (6)$$

where k_1 = retention factor of first solute and k_2 = retention factor of second solute.

The selectivity factor is calculated for each peak pair and each buffer composition according to

$$\alpha = k_2/k_1 \quad (7)$$

For each buffer composition, the software generates a list of resolutions between adjacent peaks. If the elution order is dependent on the buffer composition, the resolutions for each possible peak pair have to be calculated.

In the third step, the resolutions obtained for various buffer compositions are compared with each other. The program suggests an optimum buffer 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 analysis. The program performs an adjustment of the buffer composition for a minimum time of analysis.

Optionally, the buffer composition can be calculated where the resolution of the worst separated peak pair is the highest in the selected range regardless of analysis time. If in the selected range the maximum resolution of the worst separated peak pair (R_{max}) does not exceed the user-defined minimum, the buffer composition of R_{max} is suggested by the program as the optimum buffer composition. R_{max} corresponds to the best resolution of the worst-separated peak pair that can be obtained with SDS as surfactant and urea as modifier in the selected concentration range. If the predicted value is too low, the selectivity of the chromatographic system must be changed. This can be done by selection of another surfactant, further additives or the derivatization of solutes prior to chromatographic separation.

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

Tentatively, a second algorithm was tested that is different from the one presented only concerning the equation that was used to describe the dependence of k on c_{SDS} and c_{urea}:

$$k = f(c_{SDS}, c_{urea}) = a^* + b^* c_{SDS} + d^* c_{urea} \quad (8)$$

where a^*, b^* and d^* are constants. This equation describes in a simplified way the dependence of k on c_{SDS} and c_{urea}. The suggested buffer compositions calculated with algorithm 2, however, do not meet the requirements for a reliable optimization procedure. The results deviate strongly from those obtained with algorithm 1. The predicted buffer compositions did not allow an optimized separation concerning the test mixtures investigated. Therefore, all calculations were performed using Eq. (5) exclusively.

3.3. Urea and carbamate pesticides

The optimization software developed was applied to the separation of twelve urea and carbamate pesticides: carbofuran, phenmedipham, chlortoluron, diuron, fenuron, isoproturon, linuron, methabenzthiazuron, metobromuron, metoxuron, monolinuron and monuron. Urea and carbamate pesticides tend to decompose during gas chromatographic analysis. The main approach for their determination involves HPLC. Nine urea herbicides have already been successfully separated with MEKC and N-D-gluco-N-methylalkanamide surfactants as anionic borate complexes by Smith et al. [24].

Three test runs were performed with following separation buffers: c_{SDS} = 80 mmol/l, c_{urea} = 3.0 mol/l; c_{SDS} = 20 mmol/l, c_{urea} = 3.0 mol/l; and c_{SDS} = 20 mmol/l, c_{urea} = 0.0 mol/l. The concentrations of boric acid and disodium tetraborate were kept constant at 10 mmol/l.

As can be seen in Fig. 2a–c, co-elutions occur

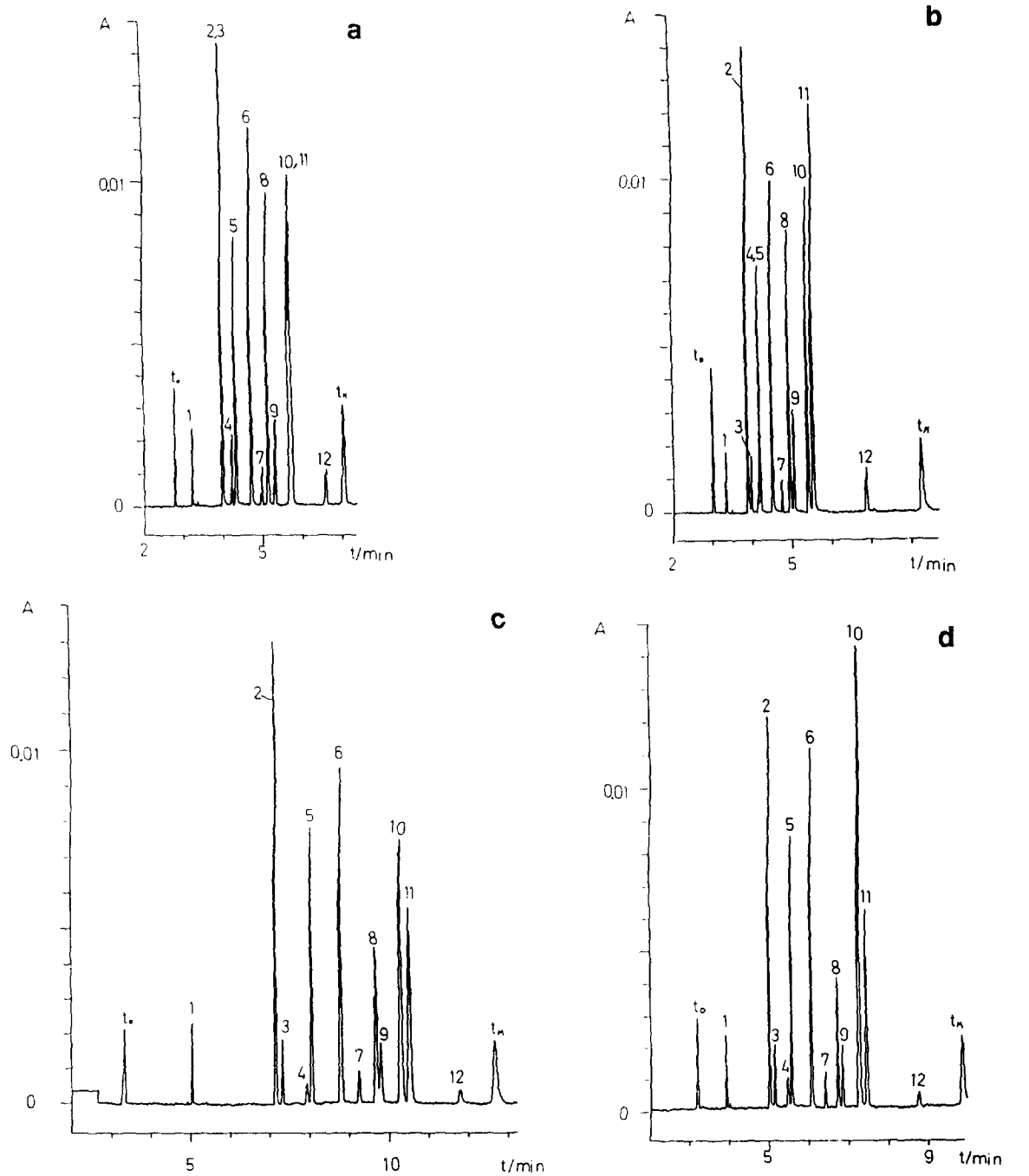

Fig. 2. Separation of twelve urea and carbamate pesticides by MEKC with various SDS and urea concentrations in the separation buffer. (a) c_{SDS} = 20 mmol/l, c_{urea} = 0.0 mol/l; (b) c_{SDS} = 20 mmol/l, c_{urea} = 3.0 mol/l; (c) c_{SDS} = 80 mmol/l, c_{urea} = 3.0 mol/l; (d) c_{SDS} = 35 mmol/l, c_{urea} = 3.25 mol/l. Solutes: 1 = fenuron; 2 = monuron; 3 = metoxuron; 4 = monolinuron; 5 = carbofuran; 6 = metobromuron; 7 = chlortoluron; 8 = isoproturon; 9 = diuron; 10 = metabenzthiazuron; 11 = linuron; 12 = phenmedipham. Capillary, 565 (500) mm × 75 μm I.D.; buffer, $c(Na_2B_4O_7)$ = 10 mmol/l, $c(H_3BO_3)$ = 10 mmol/l; voltage, 25 kV; temperature, 25°C; injection, pressure, 2.0 s; detection, photometric, 254 nm.

with all arbitrarily chosen buffer compositions. Opposite effects caused by an increase in the SDS concentration and by an increase in the urea concentration impede a rapid one-parameter optimization approach. It can be also seen that the elution window (t_0/t_M) is strongly affected by the buffer composition while the hold-up time varies only marginally (t_0 = 2.78–3.35 min).

The retention data for the three test runs were used for the prediction of the optimum buffer composition. The step width for Δc_{SDS} was fixed at 5 mmol/l and for Δc_{urea} at 250 mmol/l. The plate number was fixed at 100 000. The threshold value for the minimum resolution between each peak pair was fixed at 1.70, because the peak areas in the test solution are not equal. This threshold value was not exceeded. The maximum resolution obtainable for the worst separated peak pair is predicted to be 1.26 with a separation buffer containing 35 mmol/l SDS and 3.25 mol/l urea. In Fig. 2d, the chromatogram obtained with the optimized composition of the separation buffer is shown. In contrast to the test runs, nearly baseline resolution is achieved for all peaks.

In Table 1, the predicted retention times are compared with the retention times obtained with the suggested buffer composition. The comparison shows that the predicted retention times are overestimated with an approximately constant value of about 0.2 min. The differences between retention times of analytes eluting in succession are predicted with high accuracy. The optimization software developed generates a simulated chromatogram for the suggested buffer composition. The simulated chromatogram is depicted in Fig. 3. There is good agreement between the measured (Fig. 2d) and the simulated chromatograms.

The deviation of the predicted values from the experimentally determined retention times can be explained be the high standard deviation of the regression planes for t_0 and t_M (Fig. 1a and b). During the use of a separation capillary, sorption and swelling processes alter the surface of the fused-silica capillary. These processes can be eliminated by rinsing procedures. The influence of various rinsing procedures on the reproducibility in capillary electrophoresis has already been investigated extensively in the past [25,26]. In spite of these procedures, the surface of the capillary actually employed is altered irreversibly during its use and the capillary must be exchanged after a few months of use. The prediction of optimum conditions and of retention times in MEKC is therefore inherently hampered by the fact that the electroosmotic velocity cannot be kept completely constant. It is recommended to perform all test runs used for the prediction of optimum conditions on the same day.

3.4. Derivatized aliphatic amines

The determination of biogenic amines is of great interest in oenological studies owing to the potential toxicity of some of the aliphatic amines occurring in red and white wine [27]. At present, HPLC is the preferred method for the determination of biogenic amines in wine. We have studied the separation by MEKC of the amines methylamine, ethylamine, propylamine, isopropylamine, butylamine, 3-methylbutylamine, pentylamine, ethanolamine, morpholine, diethylamine, phenethylamine, tryptamine and 1,4-diaminobutane.

Prior to detection, a derivatization step is necessary to shift the absorption spectra of the analytes in order to permit photometric detection at longer wavelengths. Generally, *o*-phthaldialdehyde (OPA) and dansyl chloride (Dns-Cl) are used as derivatizing agents [27]. Whereas OPA reacted only with primary amines, DNS-Cl turned out to form very non-polar derivatives that cannot be separated in MEKC with SDS- and urea-containing mobile phases owing to the high retention factors of these derivatives.

P-Nitrobenzoyl chloride forms stable amides with primary and secondary amines. The nitro group of the derivatizing agent provides sufficient polarity of the formed derivative, thus rendering possible subsequent separation by MEKC with SDS- and urea-containing mobile phases. Photometric detection can be performed at 254 nm.

Three test runs were performed with following

Table 1
Predicted and measured retention times under optimized conditions

Component	Predicted retention time (min)	Measured retention time (min)	Predicted time difference between adjacent peaks (min)	Measured time difference between adjacent peaks (min)
Fenuron	3.76	3.91	1.01	1.08
Monuron	4.77	4.99	0.10	0.13
Metoxuron	4.87	5.12	0.35	0.33
Carbofuran	5.22	5.45	0.08	0.09
Monolinuron	5.30	5.54	0.49	0.50
Metobromuron	5.79	6.04	0.35	0.35
Chlortoluron	6.14	6.39	0.32	0.31
Isoproturon	6.46	6.70	0.11	0.12
Diuron	6.57	6.82	0.44	0.41
Methabenzthiazuron	7.01	7.23	0.21	0.19
Linuron	7.22	7.42	1.42	1.32
Phenmedipham	8.64	8.74	–	–
Ethanolamine	4.43	4.24	0.38	0.37
Methylamine	4.81	4.61	0.12	0.10
Morpholine	4.93	4.71	0.43	0.39
Ethylamine	5.36	5.10	0.53	0.46
Isopropylamine	5.89	5.56	0.24	0.20
By-product	6.13	5.76	0.36	0.32
Propylamine	6.49	6.08	1.24	1.04
Diethylamine	7.73	7.12	0.17	0.14
By-product	7.90	7.26	0.65	0.53
1-Butylamine	8.55	7.79	1.74	1.36
3-Methylbutylamine	10.29	9.15	0.44	0.35
Amylamine	10.73	9.50	0.25	0.19
Phenethylamine	10.98	9.69	0.66	0.54
By-product	11.64	10.23	0.47	0.45
Tryptamine	12.11	10.68	0.38	0.07
1,4-Diaminobutane	12.49	10.75	–	–
Trinitrotoluene	5.58	5.42	0.88	0.81
2,4-Dinitrotoluene	6.46	6.23	0.12	0.11
2,5-Dinitrotoluene	6.58	6.34	0.17	0.14
2,6-Dinitrotoluene	6.75	6.48	0.21	0.20
3,4-Dinitrotoluene	6.96	6.68	0.17	0.16
o-Nitrotoluene	7.13	6.84	0.10	0.11
2,3-Dinitrotoluene	7.23	6.95	0.11	0.09
p-Nitrotoluene	7.34	7.04	0.14	0.08
m-Nitrotoluene	7.48	7.12	–	–

separation buffers: $c_{SDS} = 80$ mmol/l, $c_{urea} = 3.0$ mol/l; $c_{SDS} = 20$ mmol/l, $c_{urea} = 3.0$ mol/l; and $c_{SDS} = 20$ mmol/l, $c_{urea} = 0.0$ mol/l. The concentrations of boric acid and disodium tetraborate were kept constant at 10 mmol/l.

The chromatograms obtained are shown in Fig. 4a–c. With all arbitrarily chosen buffer compositions, co-elutions occurred. *p*-Nitrobenzoic acid is one of the by-products of the derivatization procedure. The migration time of *p*-nitrobenzoic acid is virtually unaffected by the buffer composition, indicating that it does not interact with the micelles and it is retarded relative to the electroosmotic flow by its electro-

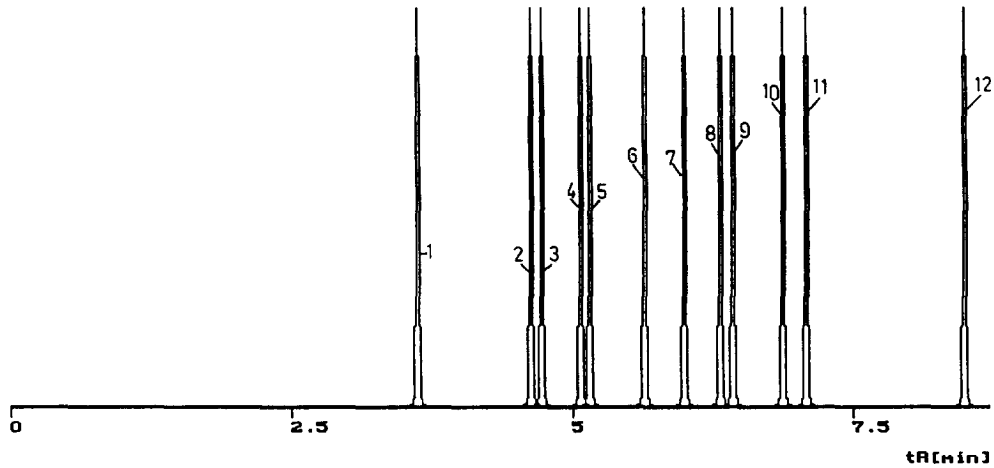

Fig. 3. Simulated chromatogram for the separation of twelve urea and carbamate pesticides under the conditions employed for the development of the chromatogram shown in Fig. 2d.

phoretic mobility. Because of its retention behavior, which differs completely from that of the solutes of interest, *p*-nitrobenzoic acid was excluded from the optimization process.

The retention data for the three test runs were used for the prediction of the optimum buffer composition. The step width for Δc_{SDS} was fixed at 5 mmol/l and for Δc_{urea} at 100 mmol/l. The plate number was fixed at 100 000. The maximum resolution obtainable for the worst separated peak pair is predicted to be 1.62 with a separation buffer containing 55 mmol/l SDS and 2.0 mol/l urea. In Fig. 4d the chromatogram obtained with the optimized composition of the separation buffer is shown. In contrast to the test runs, nearly baseline resolution is achieved for all peaks with the exception of *p*-nitrobenzoic acid, which was excluded from the optimization procedure. It co-elutes with one of the by-products of the derivatization reaction.

The two by-products can be easily separated from each other by variation of the urea concentration in the separation buffer. The resulting chromatogram is shown in Fig. 4e. It can be seen that, as predicted by the computer program, the resolution of other peak pairs is diminished on employing a separation buffer with lower urea concentration.

In Table 1, the predicted retention times for the separated amides are compared with the experimentally measured values. Whereas the predicted absolute migration times differ considerably from the measured migration times, there is good agreement for the time differences between adjacent peaks. The poor prediction of absolute migration times reflects the low day-to-day reproducibility of the electroosmotic velocity.

3.5. Nitrotoluenes

The determination of nitroaromatic compounds has become of increasing interest owing to the problem of explosives residues in soils near former ammunition plants. The concentrations of these residues are relatively high. The determination of nitroaromatic compounds in soil samples is possible by MEKC with photometric detection without preconcentration steps during sample preparation [4,28]. The large number of possible positional isomers makes their separation in reversed-phase HPLC difficult. The following congeners were successfully separated by MEKC following the procedure presented herein: 2,4,6-trinitrotoluene, 2,3-dinitrotoluene, 2,4-dinitrotoluene, 2,5-dinitrotol-

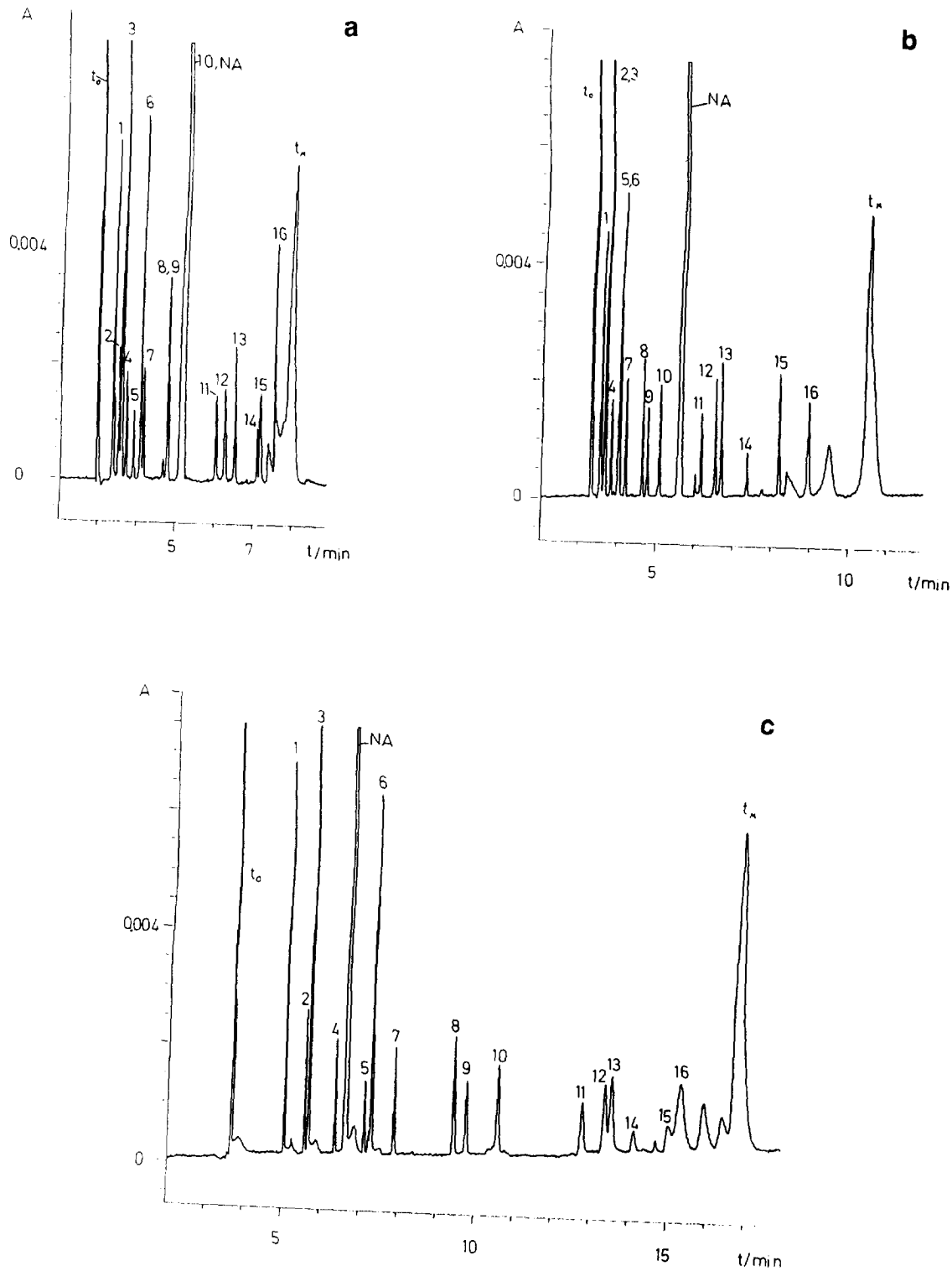

Fig. 4 (*Continued on p. 92*)

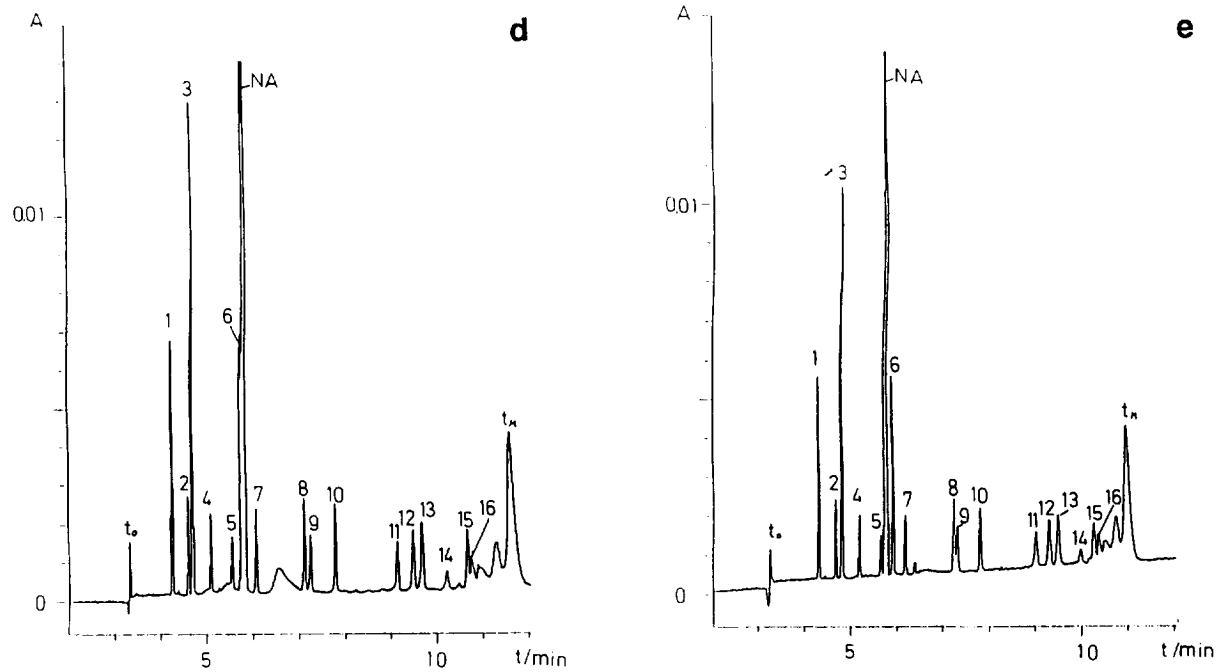

Fig. 4. Separation of thirteen aliphatic amines derivatized with *p*-nitrobenzoyl chloride by MEKC with various SDS and urea concentrations in the separation buffer. (a) $c_{SDS} = 20$ mmol/l, $c_{urea} = 0.0$ mol/l; (b) $c_{SDS} = 20$ mmol/l, $c_{urea} = 3.0$ mol/l; (c) $c_{SDS} = 80$ mmol/l, $c_{urea} = 3.0$ mol/l; (d) $c_{SDS} = 55$ mmol/l, $c_{urea} = 2.0$ mol/l; (e) $c_{SDS} = 55$ mmol/l, $c_{urea} = 1.25$ mol/l. Solutes: 1 = ethanolamine; 2 = methylamine; 3 = morpholine; 4 = ethylamine; 5 = isopropylamine, 6 = by-product; 7 = propylamine; 8 = diethylamine; 9 = by-product; 10 = 1-butylamine; 11 = 3-methylbutylamine; 12 = amylamine; 13 = phenethylamine; 14 = by-product; 15 = tryptamine; 16 = 1,4-diaminobutane. Capillary, 565 (500) mm × 75 μm I.D.; buffer, $c(Na_2B_4O_7) = 10$ mmol/l, $c(H_3BO_3) = 10$ mmol/l; voltage, 25 kV; temperature, 25°C; injection, pressure, 1.5 s; detection, photometric, 254 nm.

uene, 2,6-dinitrotoluene, 3,4-dinitrotoluene, *o*-nitrotoluene, *m*-nitrotoluene and *p*-nitrotoluene.

Three test runs were performed with following separation buffers: $c_{SDS} = 80$ mmol/l, $c_{urea} = 3.0$ mol/l; $c_{SDS} = 20$ mmol/l, $c_{urea} = 3.0$ mol/l; and $c_{SDS} = 20$ mmol/l, $c_{urea} = 0.0$ mol/l. The concentrations of boric acid and disodium tetraborate were kept constant at 10 mmol/l.

The chromatograms obtained are shown in Fig. 5a–c. Co-elutions occur with all arbitrarily chosen buffer compositions. In one case the elution order is reversed by the addition of urea to the separation buffer, although an electrophoretic velocity of the solutes can be excluded. Possibly, the addition of urea to the separation buffer affects the retention mechanism of the polar nitroaromatic compounds by the micelles. Studies of the temperature dependence of the distribution coefficient between the micellar phase and the aqueous phase show that the interaction of polar solutes with the micelles is substantially different from the interaction of non-polar solutes with the micellar phase [29]. In the case of polar solutes, the entropy change for the phase transfer of a solute from the aqueous phase into the micellar phase is negative, thus counteracting the transfer into the micellar phase. Terabe et al. [29] attributed the negative entropy change to the restricted motion of polar solutes on the surface of the micelles. This balance of forces might be influenced by urea in the counterlayer of the anionic micelles.

The retention data of the three test runs were used for a prediction of the optimum buffer composition. The step-width for Δc_{SDS} was fixed at 5 mmol/l and for Δc_{urea} at 100 mmol/l. The plate number was fixed at 100 000. The maximum resolution obtainable for the worst separated peak pair is predicted to be 1.41 with a separation buffer containing 55 mmol/l SDS and

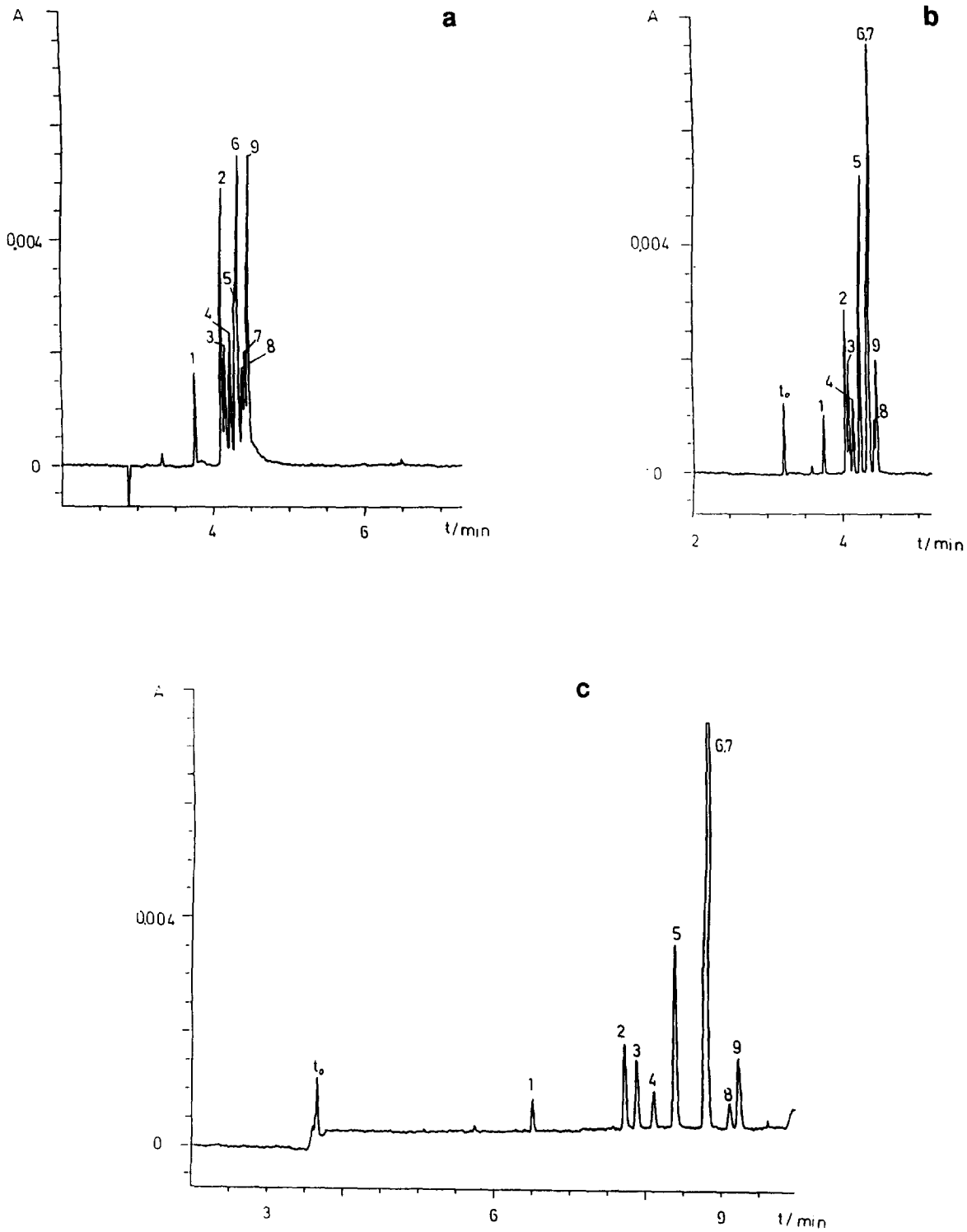

Fig. 5 (*Continued on p. 94*)

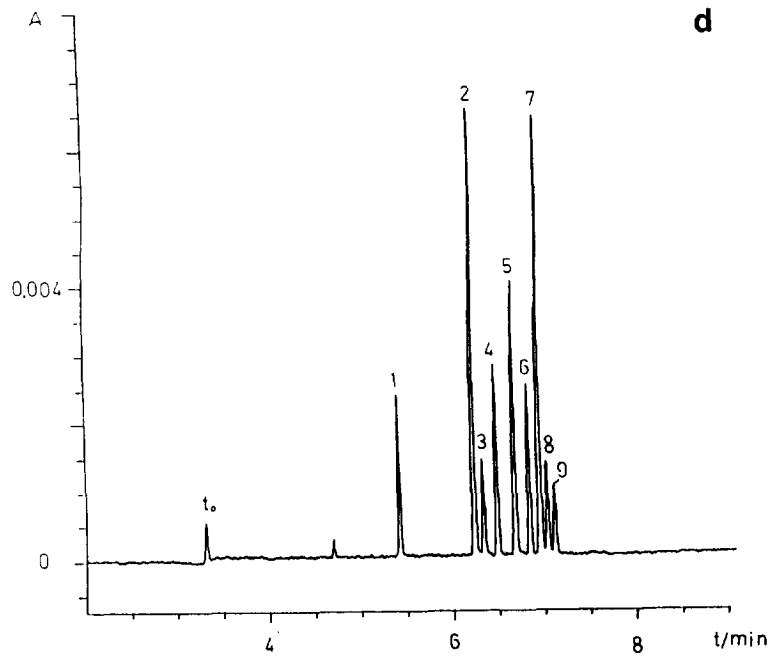

Fig. 5. Separation of nitrotoluenes by MEKC with various SDS and urea concentrations in the separation buffer. (a) c_{SDS} = 20 mmol/l, c_{urea} = 0.0 mol/l; (b) c_{SDS} = 20 mmol/l, c_{urea} = 3.0 mol/l; (c) c_{SDS} = 80 mmol/l, c_{urea} = 3.0 mol/l; (d) c_{SDS} = 55 mmol/l, c_{urea} = 1.8 mol/l. Solutes: 1 = 2,4,6-trinitrotoluene; 2 = 2,4-dinitrotoluene; 3 = 2,5-dinitrotoluene; 4 = 2,6-dinitrotoluene; 5 = 3,4-dinitrotoluene; 6 = *o*-nitrotoluene; 7 = 2,3-dinitrotoluene; 8 = *p*-nitrotoluene; 9 = *m*-nitrotoluene. Capillary, 565 (500) mm × 75 μm I.D.; buffer, $c(Na_2B_4O_7)$ = 10 mmol/l, $c(H_3BO_3)$ = 10 mmol/l; voltage, 25 kV; temperature, 25°C; injection, pressure, 1.0 s; detection, photometric, 254 nm.

1.80 mol/l urea. In Fig. 5d, the chromatogram obtained with the optimized composition of the separation buffer is shown. In contrast to the test runs, nearly baseline resolution is achieved for all peaks. In Table 1 the predicted retention times for the optimized buffer composition are compared with the retention times obtained experimentally with this buffer composition. The comparison shows that the differences between retention times of analytes eluting in succession are predicted with high accuracy, although the predicted retention times are slightly overestimated.

The optimization of the separation of nitrotoluenes shows that the approach used herein for the optimization of the buffer composition can cope with a high dependence of the selectivity coefficients on the buffer composition and reversals of the elution order.

4. Conclusions

It was shown that the composition of the separation buffer (with regard to the SDS and urea concentrations) can be rapidly optimized for a complex mixture of solutes by the developed computer-aided procedure. It is possible to predict the migration times of neutral solutes in MEKC on the basis of only three initial experiments. It was demonstrated that urea is an ideal modifier, because it extends the elution range without reducing substantially the electroosmotic velocity.

This study involved only two factors (SDS and urea concentrations). The concept, however, is entirely general and can be extended to other capillary electrophoretic systems such as MEKC with cationic micelles or cyclodextrin-modified capillary electrophoresis.

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