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Systematic optimization of peak capacity for pesticide separation in micellar electrokinetic chromatography

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Abstract

A systematic study for separating seven triazine pesticides was undertaken on the basis of micellar electrokinetic chromatography. The effects of five experimental factors (voltage, temperature and concentrations of buffer, sodium dodecylsulfate concentration, and acetonitrile) on the migration time, peak width, plate number and peak capacity were studied. Optimization was based on screening and a response surface designs. The study has shown that especially the peak width strongly varies in dependence on the experimental conditions. Therefore, common chromatographic measures for calculating the plate number and the peak capacity cannot be directly related to the experimental variables. Quantitative modelling of objective criteria, such as plate number and peak capacity on experimental factors is only possible if individual models are computed for each solute with respect to its migration time and peak width. On the basis of individual solute migration models reliable plate numbers and peak capacities were derived and used for evaluation of the optimum separation conditions.

Keywords: Optimization; Mathematical modelling; Experimental design; Response surface modelling; Chemometrics; Pesticides; Triazines

1. Introduction

Analysis of constituents in highly complex samples, such as soil extracts, requires separation methods of high peak capacity. Especially in the case of liquid-phase analytical separations, the required peak capacities very often cannot be attained by the existing HPLC based separations. Capillary electrophoresis (CE) in its different versions is predestined to fill this gap because of its high efficiency and resolution.

Several papers on optimizing efficiency and resolution in CE have been reported based on either univariate [1,2] or multivariate approaches [3–6]. In more mechanistic approaches migration behaviour of individual solutes or their resolution is described by physico-chemical models as a function of experimental factors [7,8].

Problems arise from the complexity of those separations, thus in the separation of peptides in Ref. [4] only part of the experiments could be used for building a mathematical model. In Ref. [5] direct modelling of different objective functions on the experimental variables revealed rather poor fits. As the objective functions, most frequently plate number and resolution are used. Nielsen et al. [3] applied the number of appearing peaks as the objective criterion.

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Until now, no attention has been paid to the optimization of peak capacity.

Definite conclusions can only be drawn, if a good mathematical model is found to describe the experimental data. Therefore, in this work systematic optimizations in CE are reconsidered with the aim to approximate migration data as close as possible by a mathematical model and to include a measure for the peak capacity as an objective criterion. The procedure is explained for separating triazine pesticides by means of micellar electrokinetic (capillary) chromatography (MEKC).

2. Experimental

2.1. Chemicals

The triazine pesticide mixture was acquired from Supelco, catalogue No. 4-8392, containing the compounds simazine, atrazine, prometon, ametryn, propazine, prometryn and terbutryn. A $5 \cdot 10^{-6}$% (w/w) amount of each of the seven pesticides were dissolved in water. All solutions were prepared by using redistilled water. Sodium hydrogen phosphate and sodium tetraborate decahydrate were purchased from Merck (Darmstadt, Germany) and sodium dodecyl sulfate (SDS) was from Feinchemikalien Sebnitz. All chemicals were of analytical reagent grade. The composition of phosphate/borate buffer was adjusted at molar ratios of 3 to 2, respectively.

2.2. Apparatus

All experiments were carried out on a HP ^{3D}Capillary Electrophoresis System (Hewlett-Packard, Waldbronn, Germany) controlled by the HP ChemStation software. The detection was performed by using a diode array detector at the wavelength of 214 nm and a bandwidth of 20 nm. All separations were done on standard capillaries, 50 μm I.D., delivered by Hewlett-Packard or Supelco. The total and effective length were 64 and 56 cm, respectively. All injections were done by pressure at 120 mbar×s from a synthetic sample solution in pure water.

An electropherogram as the starting point of the opimization is given in Fig. 1. Identification of peaks was performed by injection of a solution of a single solute and comparison of migration times. The starting conditions were taken from an application note [9].

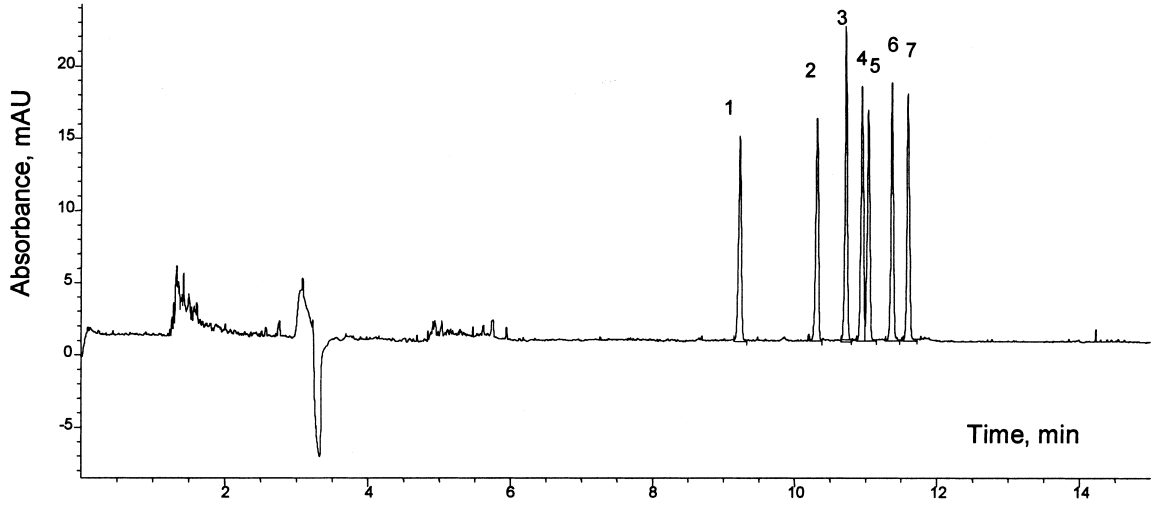

Fig. 1. Starting separation: 20 m*M* phosphate-buffer (3:2 molar ratio), 100 m*M* SDS, pH 9.14, voltage 30 kV, temperature 25°C, 64 cm (56 cm effective length)×50 μm I.D. capillary, injection 150 mbar/s, detection at 214 nm, each analyte at $5 \cdot 10^{-6}$ %(w/w) in aqueous solution. (1 – Simazine, 2 – atrazine, 3 – prometon; 4 – ametryn, 5 – propazine, 6 – prometryn, 7 – terbutryn).

2.3. Software

Numerical determinations of peak widths and areas were performed with MATHEMATICA for Students V 2.2 (Wolfram Research). Mathematical modelling was carried out with the software MATLAB 5.0 (MathWorks).

3. Results and discussion

3.1. Experimental design

Systematic optimization was based on running experimental designs at two and three factor levels. The results were visualized by plotting response surfaces of the optimization criteria in dependence on the factors studied. To decide on the most important factors in the MEKC separation of the seven pesticides a screening design was run in the first step. This design was based on a two-level full factorial design for the following five factors: buffer concentration, SDS concentration, acetonitrile (ACN) content as organic modifier, temperature and voltage. The adjustment of the five factors and the results for the migration times of the last peak and for the plate numbers are given in Table 1. The plate number per meter was calculated according to:

$$N/\mathrm{m} = 5.54 \cdot \left(\frac{t_{m,\mathrm{last}}}{w_{0.5,\mathrm{last}}} \right)^2 \Big/ L_{\mathrm{eff}} \qquad (1)$$

where $t_{m,\mathrm{last}}$ and $w_{0.5,\ \mathrm{last}}$ are migration time and peak width at half maximum of the last eluting peak, respectively. L_{eff} represents the effective length, i.e. 56 cm.

From Table 1 it is obvious that in the case of too high concentrations of both buffer and SDS the time system limit is reached which was fixed at 70 min total analysis time. The analysis time becomes too large because of a low electroosmotic flow (EOF) caused by very high adsorptions of sodium cations at the negatively charged wall. The other effect of too high buffer concentrations combined with too low SDS concentrations is that an enormous peak dispersion occurs. The result is a high current (data not shown) with the consequence that in some cases already the fourth analyte could not be detected any more because its peak was too much broadened (runs 22 and 26 in Table 1).

From the measurements on the basis of the screening design the factor effects can be described as follows:

Too high buffer concentrations cause tremendous peak dispersion. Thus, the buffer concentration, studied here in the range from 20 to 50 m*M*, should not be too high. From the experiments it could be expected that by decreasing the buffer concentration below 20 m*M* the efficiency could be even raised and the analysis time shortened.

Increasing the SDS concentration improves the efficiency but also extends analysis time and leads to peak dispersion probably due to Joule heating. Therefore, the concentration of SDS should not exceed 50 m*M*.

The addition of ACN as an organic modifier widens the chromatographic window while reducing the EOF. So the studied range between 0 and 20% ACN was further included in the systematic study. Sometimes, however, ACN concentrations above 10%(v/v) may cause problems by air bubble formation.

An increase of the temperature of the capillary from 25 to 45°C reduces somewhat the plate number but not the separation performance (N/min). A possible explanation for the reduction of the plate number is the increase in the length of the starting zone due to lower viscosity of the liquid in the capillary. The total analysis time can be decreased by one third if the higher temperature is used.

As the best suited voltage a value of 30 kV was found. Using high voltage combined with low buffer concentrations leads to yet acceptable current values below 80 μA.

Based on the aforementioned results a three-level design was set up to further optimize the separation. As factors only the concentrations of buffer, SDS and ACN were chosen while the temperature was fixed at 45°C and the voltage at 30 kV. The design was based on a central composite design [10] the factor levels of which are given in Table 2. The design was composed of a two-level full factorial design and a star design. The factor levels in the star points were positioned at the areas of the hypercube of the two-level design rather than at a hypersphere.

Three replicates were performed in the center of

Table 1
Two-level experimental design

Run	Buffer (m*M*)	SDS (m*M*)	ACN (%, v/v)	Temperature (°C)	Voltage (kV)	$t_{m,last}$ (min)	N/m $\times 10^3$
Low (−)	20	30	0	25	20		
High (+)	50	100	20	45	30		
1	−	−	−	−	−	14.961	1871
2	+	−	−	−	−	53.580	484
3	−	+	−	−	−	23.490	906
4	+	+	−	−	−	Time system limit[a]	
5	−	−	+	−	−	12.887	780
6	+	−	+	−	−	28.995	417
7	−	+	+	−	−	49.583	276
8	+	+	+	−	−	Time system limit[a]	
9	−	−	−	+	−	10.444	759
10	+	−	−	+	−	28.835	561
11	−	+	−	+	−	15.872	519
12	+	+	−	+	−	62.763	383
13	−	−	+	+	−	7.657	546
14	+	−	+	+	−	35.606	381
15	−	+	+	+	−	29.068	438
16	+	+	+	+	−	Not all peaks detectable	
17	−	−	−	−	+	9.299	890
18	+	−	−	−	+	23.998	756
19	−	+	−	−	+	12.941	1106
20	+	+	−	−	+	44.135	529
21	−	−	+	−	+	8.665	1034
22	+	−	+	−	+	Not all peaks detectable	
23	−	+	+	−	+	26.623	481
24	+	+	+	−	+	Power instable	
25	−	−	−	+	+	6.581	708
26	+	−	−	+	+	Not all peaks detectable	
27	−	+	−	+	+	9.335	489
28	+	+	−	+	+	Power system limit[b]	
29	−	−	+	+	+	6.429	698
30	+	−	+	+	+	13.370	376
31	−	+	+	+	+	15.728	528
32	+	+	+	+	+	Power system limit[b]	

[a] The time system limit was set to 70 min.
[b] The power system limit was limited by ChemStation software at 6 W.

the design. Runs 18 and 19 constitute two additional replicates. Table 2 again contains the migration of the last eluting peak and the plate number calculated by Eq. (1).

As a measure for the peak capacity later on the number of possible peaks (NPP) was used based on the formula given in Ref. [11]:

$$\mathrm{NPP} = \frac{t_{m,\mathrm{last}} - t_{m,1}}{w_{\mathrm{last}} - w_1} \cdot \ln\left(\frac{w_{\mathrm{last}}}{w_1}\right) \qquad (2)$$

where w is the peak width at its base, index 1 denotes the first peak and index 'last' the last one. In practice the peak width at half maximum has been used as generated in the HP ChemStation software. Therefore Eq. 2 has been modified as given below in Eq. 5.

3.2. Response surface modelling

Direct modelling of the plate number (Eq. (1)) or the number of possible peaks (Eq. (2)) on the factor levels gave rather poor fits. This is reasoned by a high variability of the peak width as found within a

Table 2
Three-level experimental design

Run	Block	Buffer (m*M*)	SDS (m*M*)	ACN (%,v/v)	$t_{m,\text{last}}$ (min)	$N/\text{m}\times10^3$
Low (−)		5	20	0		
Mid (0)		12.5	35	10		
High (+)		20	50	20		
1	1	−	−	−	5.074	439
2	1	−	−	+	3.505	260
3	1	−	+	−	6.194	312
4	1	−	+	+	5.817	531
5	1	+	−	−	5.868	500
6	1	+	−	+	3.847	513
7	1	+	+	−	7.131	611
8	1	+	+	+	6.723	507
9	1	0	0	0	7.708	766
10	1	0	0	0	7.753	1105
11	2	0	0	0	7.803	1379
12	2	−	0	0	7.166	529
13	2	+	0	0	9.418	1008
14	2	0	−	0	6.049	837
15	2	0	+	0	9.307	1055
16	2	0	0	−	6.620	1536
17	2	0	0	+	5.934	768
18	2	0	0	0	7.750	1228
19	2	0	0	–	6.662	1467

single run as well as between runs at different factor levels. Fig. 2A demonstrates the variation of peak width for the two-level screening design including all seven solutes. This effect is even more pronounced in the measurements based on the three-level design (Fig. 2B). Consider, e.g., runs 9, 10 and 11 in Table 2 that were generated in the center of the three-level design. Here the decrease in buffer concentration from 20 m*M* in run 8 to 12.5 m*M* in the following runs decreases the repeatability of determining the plate number. Even flushing with 0.1 *M* NaOH for 5 min after each run and flushing with back ground electrolyte for 4 min before each run, the buffer concentration has a strong effect on the equilibria inside the capillary.

Thus, the uncritical use of the formula for the plate number in Eq. (1) does not make sense. Also the increase in the peak width from low to high migration times as implied in all measures for the peak capacities is not found in all experiments (cf. Fig. 2B). Therefore, application of the formula for the number of possible peaks according to Eq. (2) is not feasible either.

The variability of the peak width, however, is not due to random effects or due to imprecise measurements but originates in the concrete experimental conditions. It can be shown that modelling of peak width in dependence on the factor levels reveals acceptable results. In this study we used polynomial models of the general form:

$$y = b_0 + \sum_{i=1}^{p} b_i x_i + \sum_{1\le i<j}^{p} b_{ij} x_i x_j + \sum_{i=1}^{p} b_{ii} x_i^2 \qquad (3)$$

where y is the response, i.e. peak width or migration time or a chromatograpic measure, b_0 is the overall mean, b_i are the regression parameters and x_i represents the p main factors. In the case of the two-level designs in Table 1 linear and interaction terms have been included into the model for the factors buffer, SDS, ACN, temperature and voltage. In the case of the three-level design also quadratic effects were considered. The significance of the parameters was evaluated by partial F-tests.

The results of modelling peak width and migration time in dependence on the factor levels of the two- and three-level designs are given in Figs. 3 and 4, respectively. One should notice that the range of

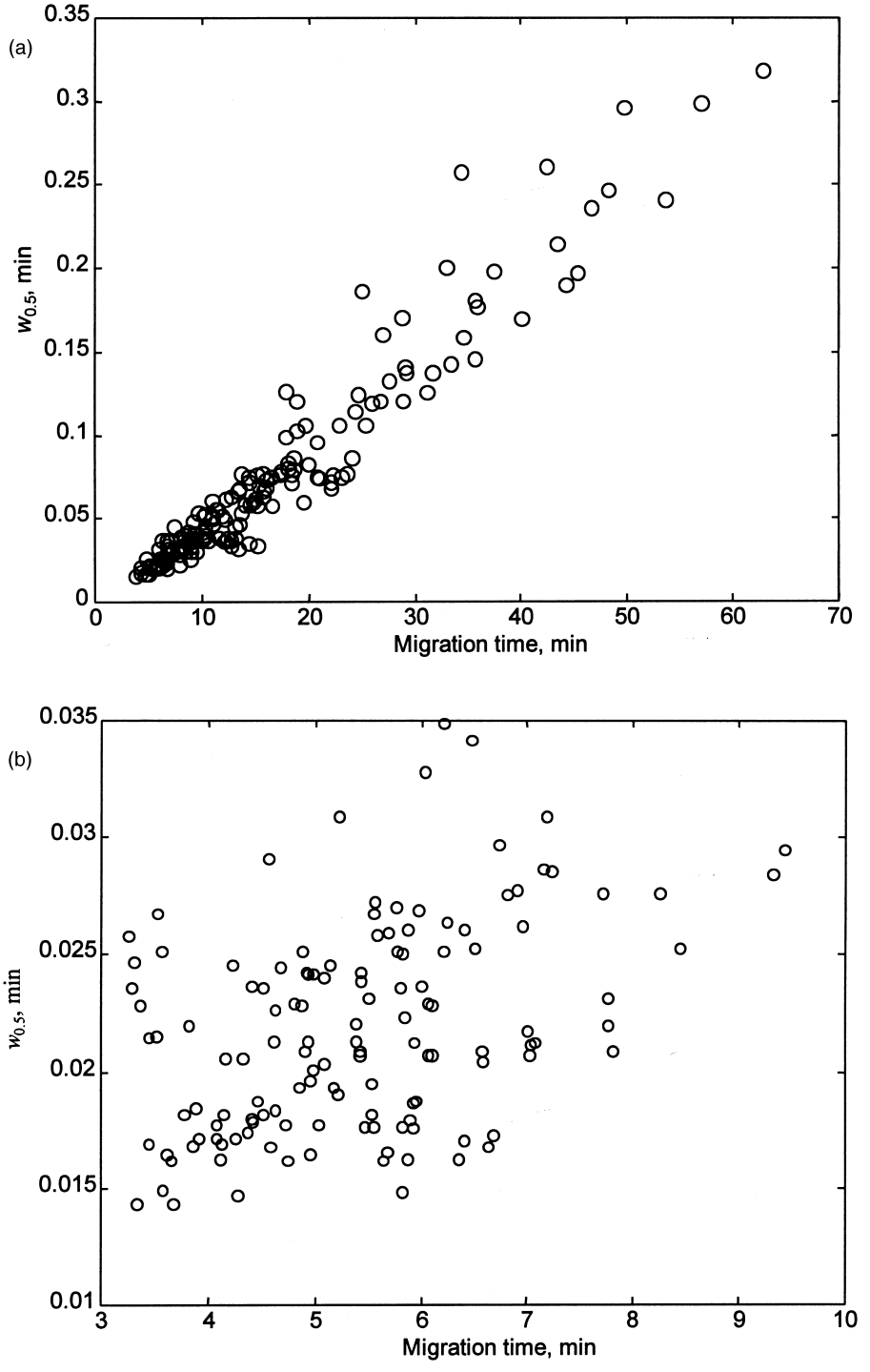

Fig. 2. (A) Variation of the peak width at half maximum, $w_{0.5}$, with migration time of seven pesticides measured for the two-level design given in Table 1. (B) Variation of the peak width at half maximum, $w_{0.5}$, with migration time of the seven pesticides measured for the three-level design given in Table 2.

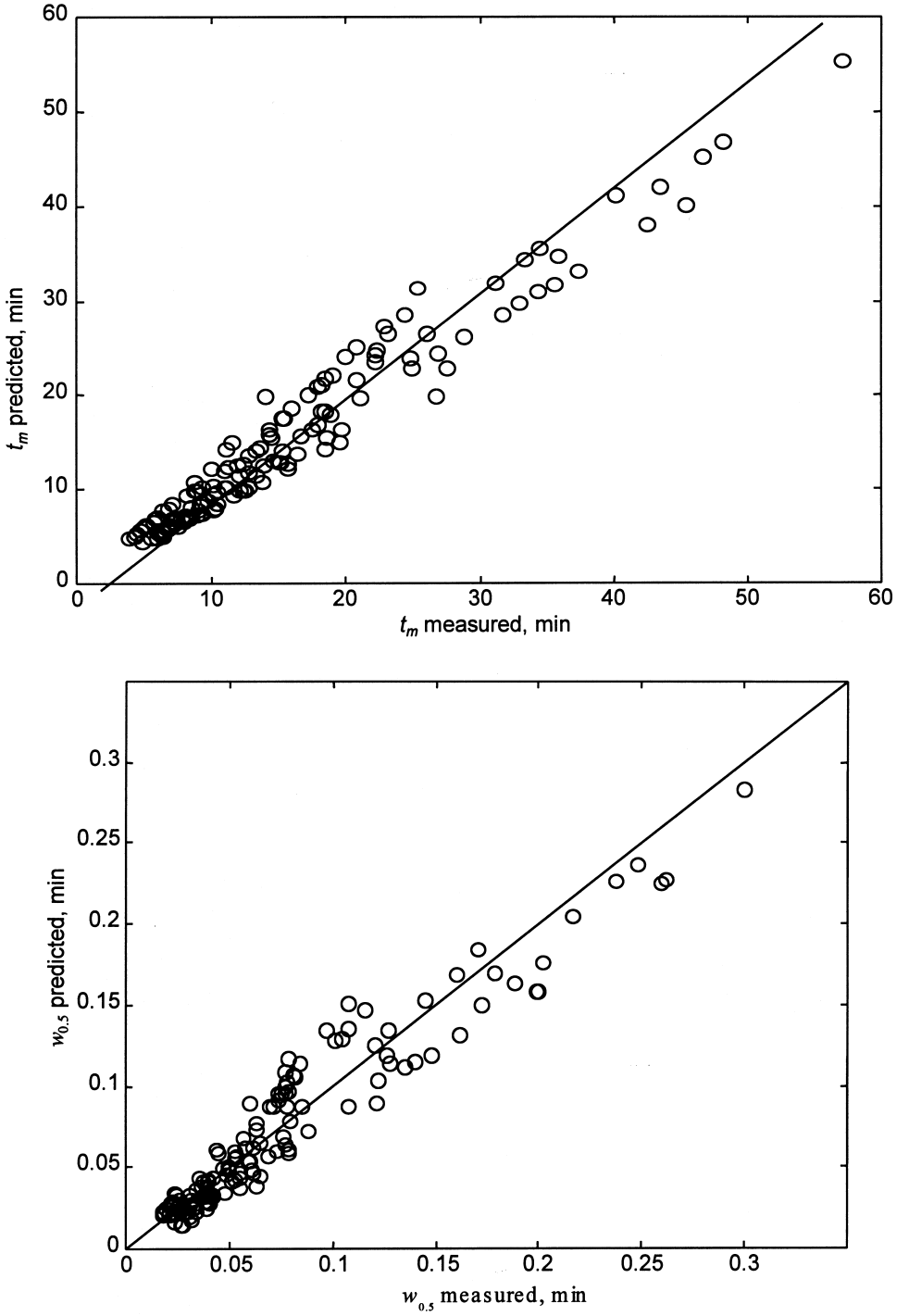

Fig. 3. Response plots for modelling migration time (top) and peak width (bottom) on the basis of the two-level design according to Table 1.

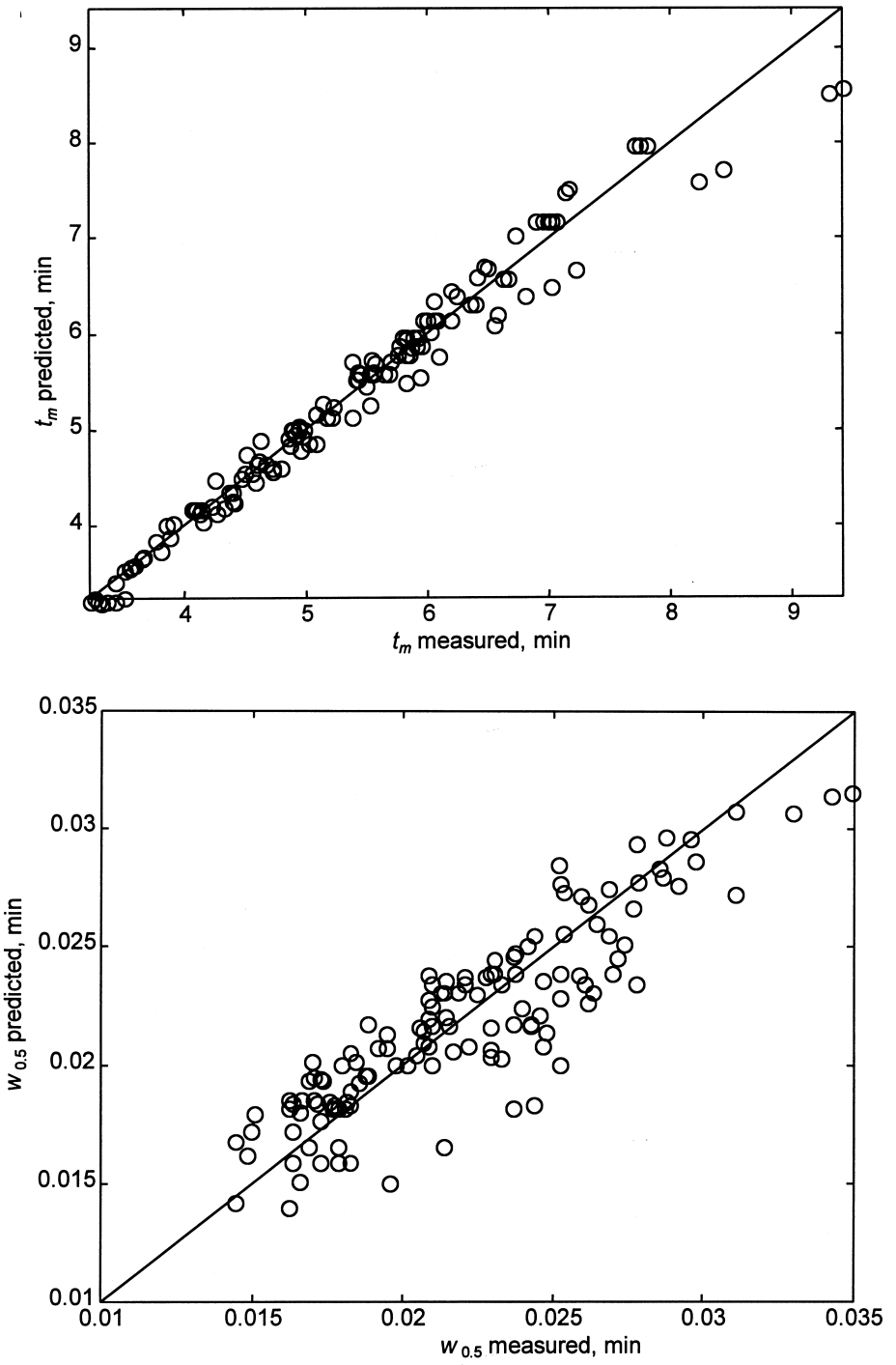

Fig. 4. Response plots for modelling migration time (top) and peak width (bottom) on the basis of the three-level design according to Table 2.

peak widths is much smaller in the three-level design experiments compared to those in the two-level design. The residual error between measured and predicted responses was evaluated on the basis of the root mean squared error (RMSE) as follows:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}(y_{ij}-\hat{y}_{ij})^2}{nm}} \quad (4)$$

where y_{ij} is the measured response for solute i of run j, $\hat{y}$ is the predicted response, n is the number of solutes and m the number of runs. The results on the residual errors are summarized in Table 3. Relative errors are 7.36% for migration time and 3.61% for peak width based on the two-level design as well as 10.69% for migration time and 8.16% for peak width for the three-level design. The complicated dependences of peak width on the concentrations of buffer, SDS, and ACN are demonstrated in Figs. 5 and 6 for the first eluting compound simazine and the last one terbutryn. Also the migration time is heavily dependent on the polarity of the molecule as demonstrated in Fig. 7.

These results of modelling migration time and peak width are an appropriate basis for optimizing efficiency and peak capacity of the separation. In contrast to former approaches, however, the chromatographic measures are not directly related to the experimental variables but at first migration time and peak width are described in separate models in dependence on the factors. Then the corrected migration time and peak width are used to calculate the objective criteria plate number and peak capacity.

The final optimization has been based on the three-level design. Typical response surface plots for the plate number and the number of possible peaks as a measure for the peak capacity are given in Fig. 8. Of course, calculating the NPP value according to Eq. (2) does not make sense, because it is not guaranteed that the width of the last peak is different from that of the first peak. Also it happens that the width of the last peak is of the same order of magnitude as that of the first peak. This phenomena can be attributed to the fact that diffusion of micelles is about one order of magnitude lower than diffusion in the aqueous phase. As a result, solutes with a high retention factor have lower diffusion coefficients and, therefore, peak broadening is less pronounced than expected from chromatographic theory. Then, of course, Eq. (2) might produce an infinite number of possible peaks. As a more adequate measure here is the averaged peak width, $\overline{w}_{\alpha 5}$, in a given run. Eq. (2) is modified therefore in the following way:

$$\mathrm{NPP} = 1 + \frac{t_{m,\mathrm{last}} - t_{m,1}}{1.699\overline{w}_{0.5}} \quad (5)$$

The factor 1.699 corrects for the measurement of peak width at half maximum rather then at its base (cf. Ref. [11]).

The quality of predicting the number of possible peaks and the plate number can be seen from the response plots in Figs. 9 and 10, respectively. In those figures the NPP value and the plate number is compared for the case of direct modelling of objective functions on the factor levels and for modelling migration time and peak width preliminary to calculating the objective criteria. The improvements reached with using the latter approach can clearly be seen.

The highest peak capacity is found in the center of the three-level design with a predicted NPP value of 111 (cf. Fig. 9). The exact experimental conditions can be best derived from a contour plot as given in Fig. 11. An electropherogram run at optimum conditions is depicted in Fig. 12. The separation time has decreased from nearly 12 to less then 8 min (cf. Fig. 1). The base line separated constituents are more equally spaced and the practically found NPP value amounts up to 104 in the migration window considered between the first and last occurring peak. Of course, to determine the peak capacity of the whole separation system one would need to extrapolate the calculation to the range between the hold-up time and the elution time of the micelles.

Table 3
Residual errors for modelling migration time and peak width in dependence on the factor levels in the two- and three-level experimental designs

Design	RMSE for t_m in min	RAISE for $w_{0.5}$ in min
Two-level	2.208	0.0160
Three-level	0.2 17	0.00204

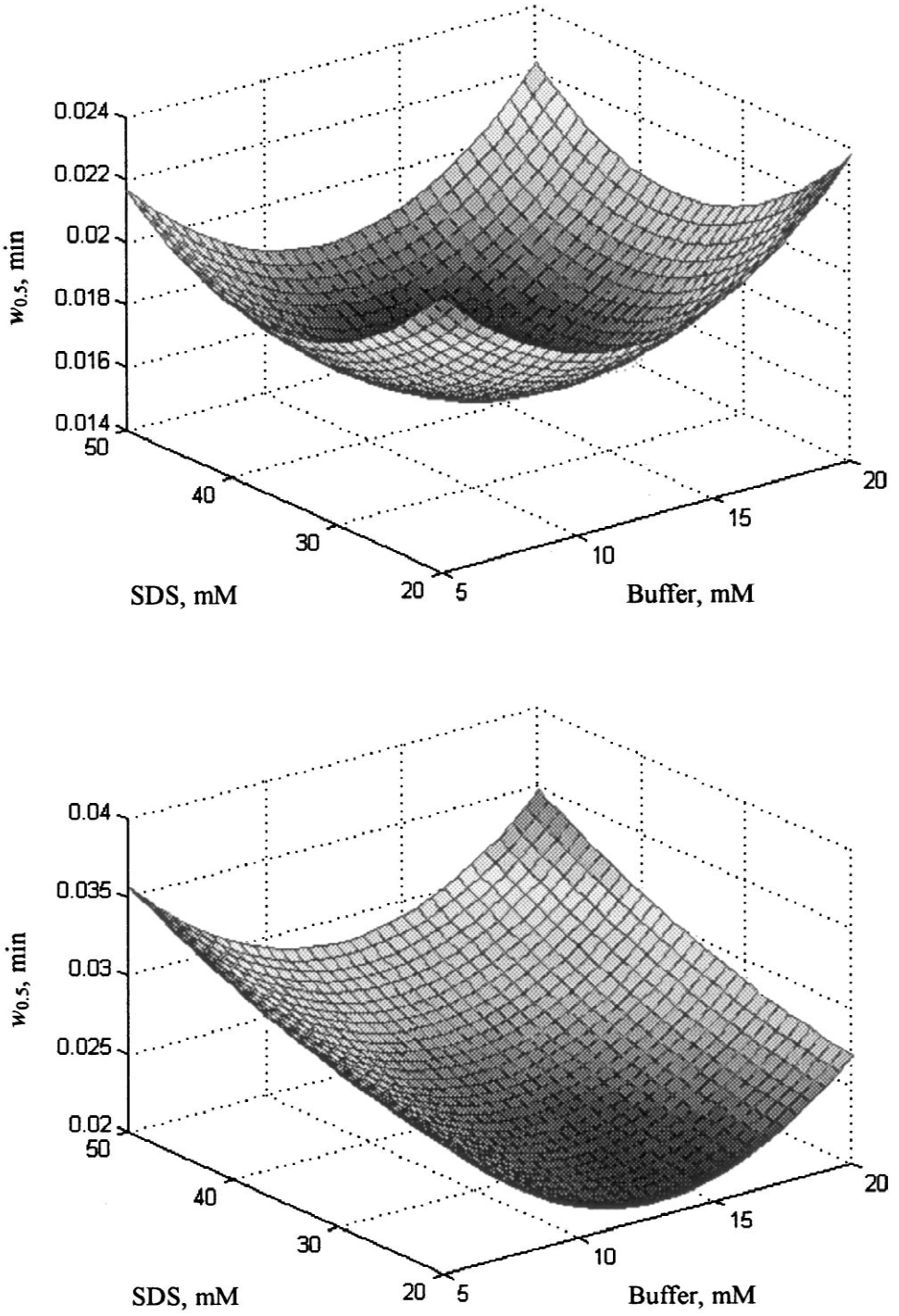

Fig. 5. Response surface plot for the peak width of simazine (top) and terbutryn (bottom) at 10%(v/v) ACN based on the three-level design according to Table 2.

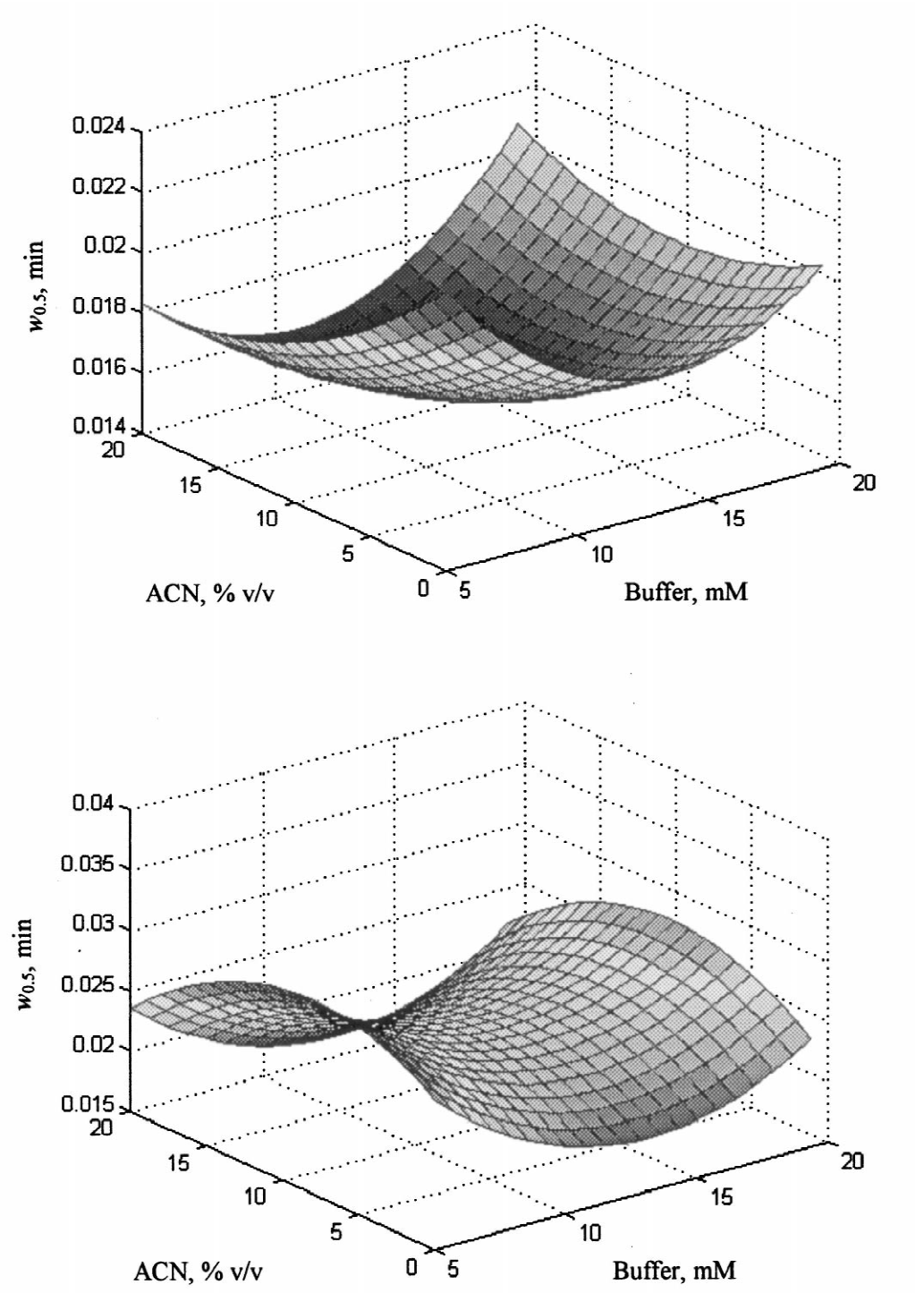

Fig. 6. Response surface plot for the peak width of simazine (top) and terbutryn (bottom) at 35 m*M* SDS based on the three-level design according to Table 2.

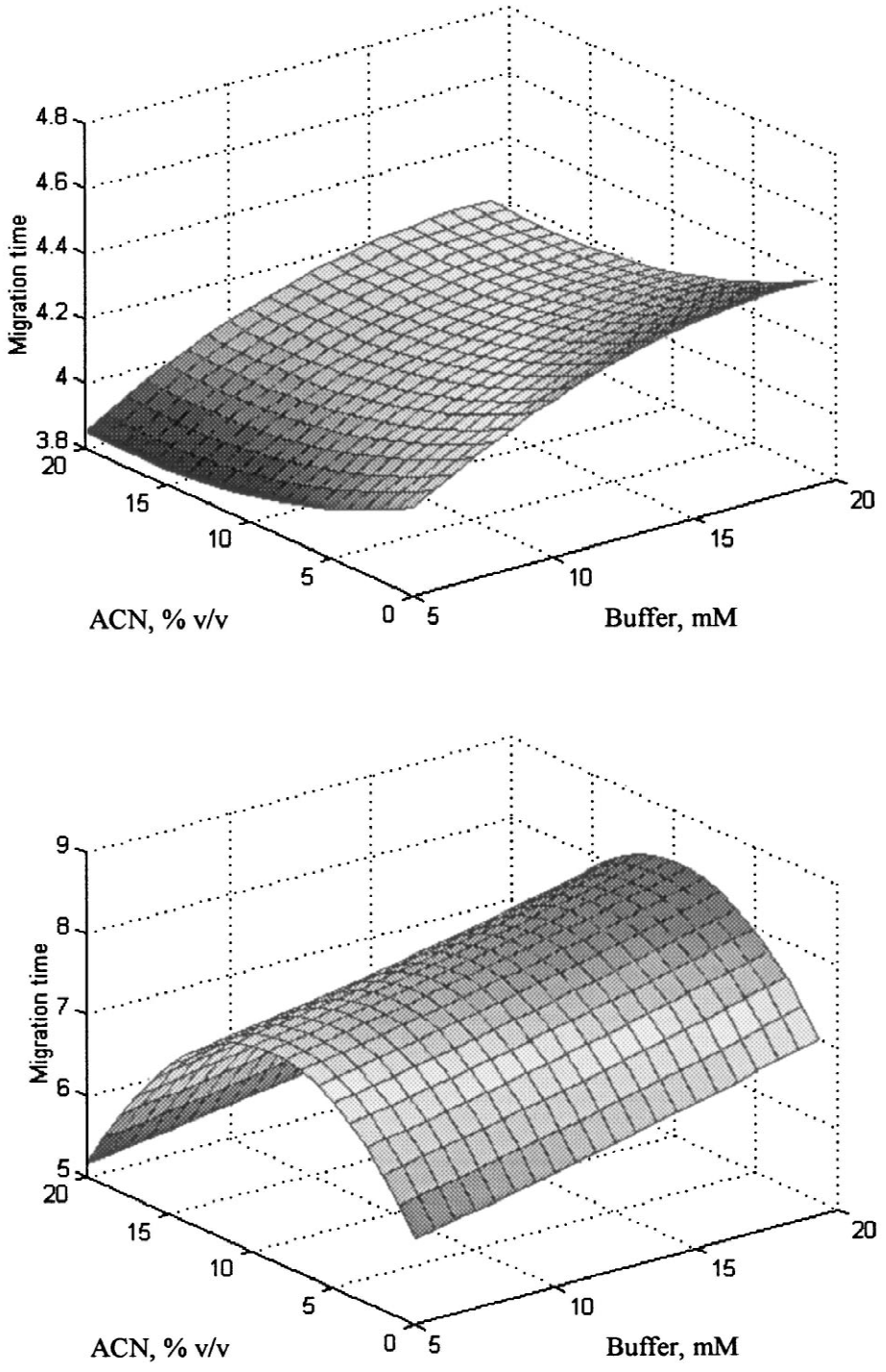

Fig. 7. Response surface plot for the migration time (given in min) of simazine (top) and terbutryn (bottom) at 35 m*M* SDS based on the three-level design according to Table 2.

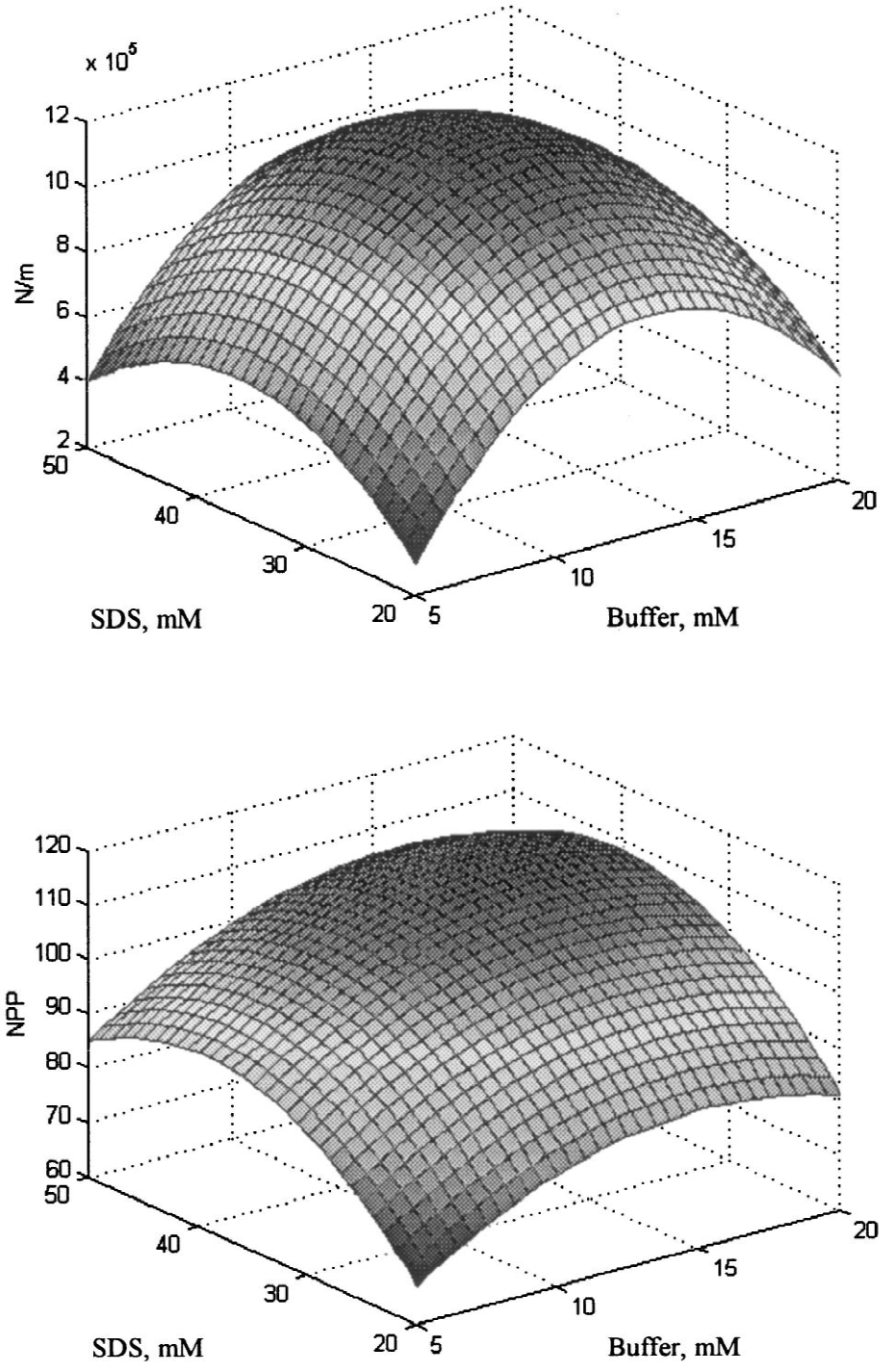

Fig. 8. Response surface plots for the plate number (top) and the number of possible peaks (bottom) at 10%(v/v) ACN based on the three-level design according to Table 2.

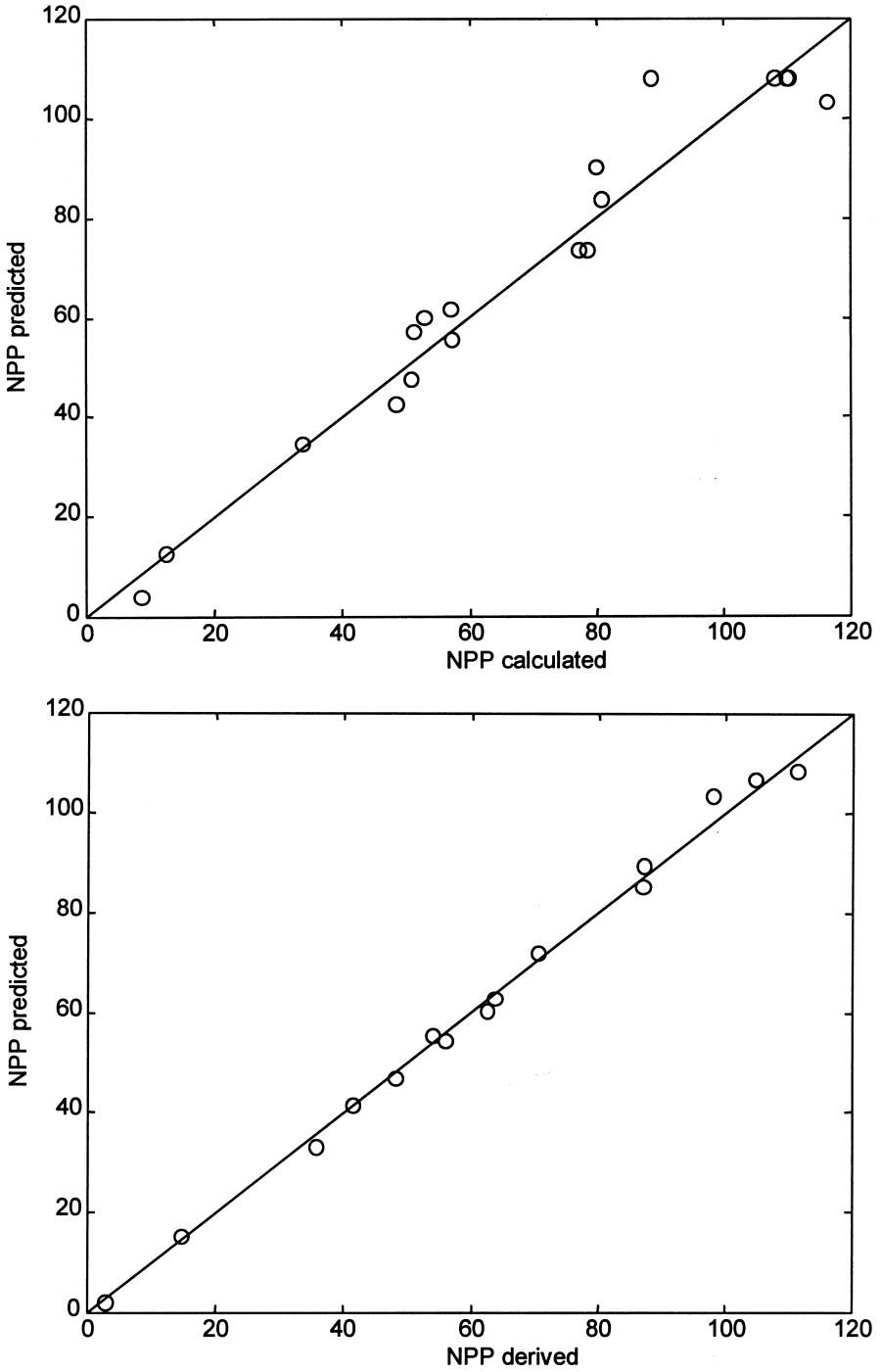

Fig. 9. Response plots for predicting the number of possible peaks on the basis of direct modelling of NPP on the factor level (top) and by calculating the NPP value from previously modelled migration times and peak widths (bottom) for the three-level design according to Table 2.

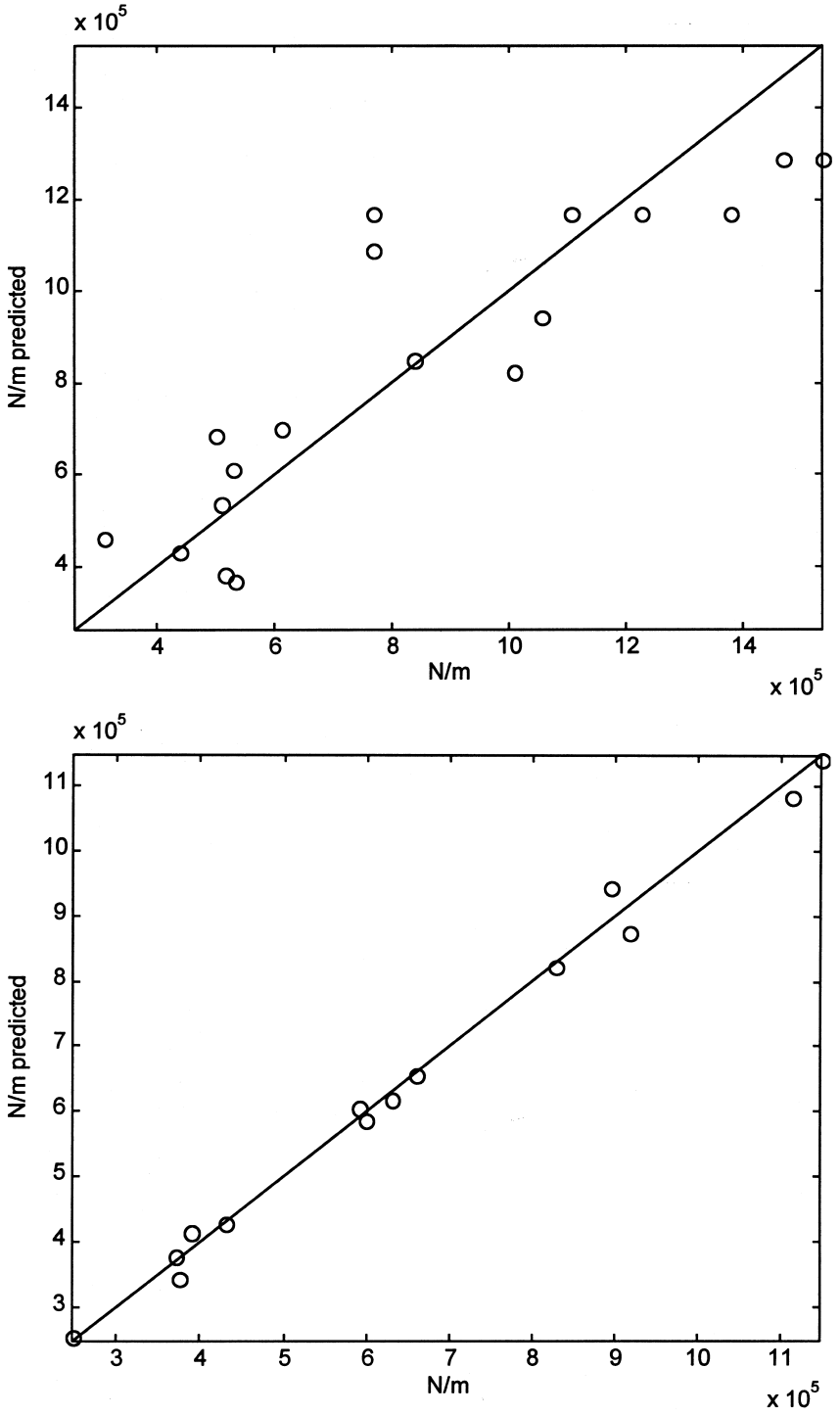

Fig. 10. Response plots for predicting the plate number on the basis of direct modelling of the plate number on the factor level (top) and by calculating the plate number from previously modelled migration times and peak widths (bottom) for the three-level design according to Table 2.

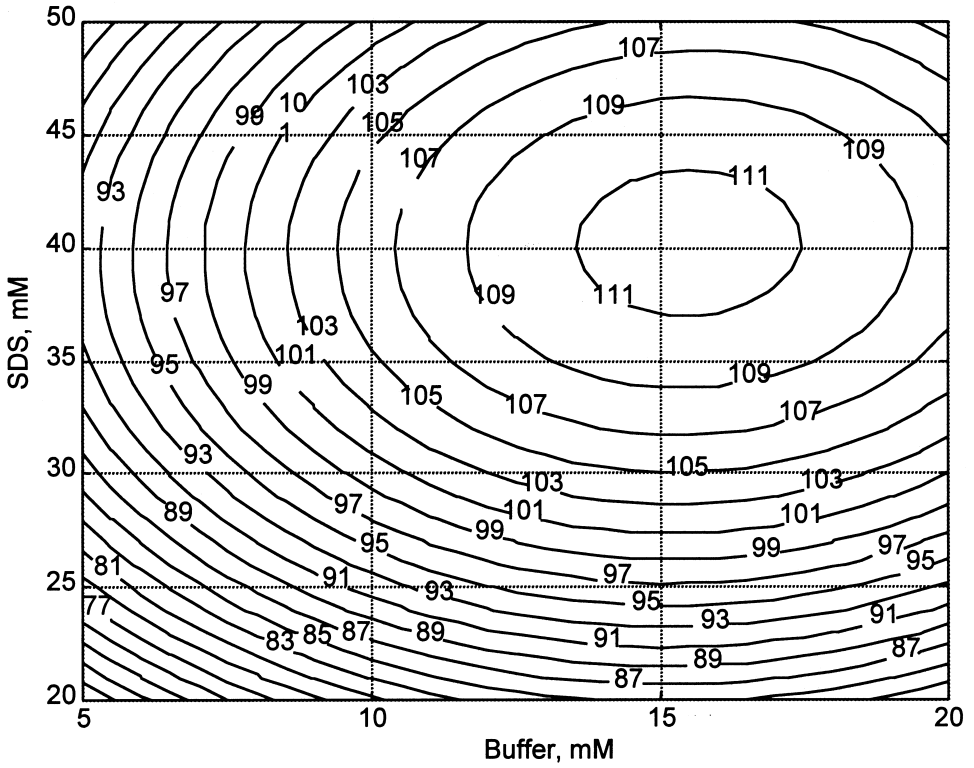

Fig. 11. Contour plot for predicting the peak capacity (NPP isolines) on the basis of previously modelled migration times at 10%(v/v) ACN for the three-level design according to Table 2.

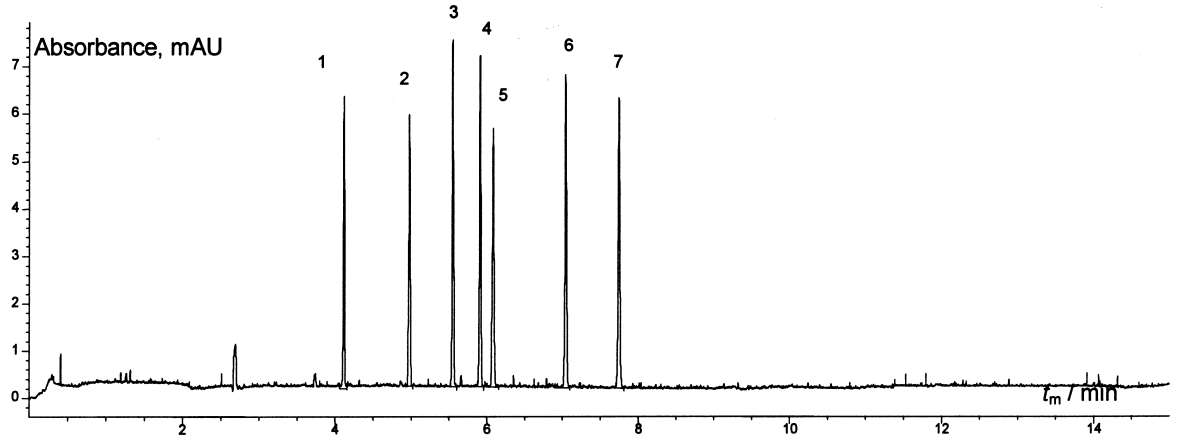

Fig. 12. Optimized run – 16.5 m*M* phosphate–borate (3:2), 40 m*M* SDS, 10%(v/v) ACN, pH=9.14, temperature 45°C, voltage 30 kV, 64 cm (56 cm effective length)×50 μm I.D. standard capillary. (1 – Simazine, 2 – atrazine, 3 – prometon; 4 – ametryn, 5 – propazine, 6 – prometryn, 7 – terbutryn).

4. Conclusions

The rather complicated dependences of peak width and retention time in MEKC on the experimental variables, such as the concentrations of buffer, SDS and ACN, hinder direct modelling of plate number and peak capacity on these variables. Instead, individual models have to be constructed for each solute with respect to its peak width and migration time. On the basis of individual solute models reliable predictions of the separation efficiency and of the peak capacity can be guaranteed. More investigations will be necessary, however, to elucidate the fundamental reasons for the high variability of the peak width in dependence on experimental conditions as compared to common chromatographic separation methods.

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