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Selectivity optimization for the separation of chlorophenols in an irregularly shaped experimental region in capillary electrophoresis

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Abstract

The separation of seventeen chlorophenol congeners and phenol was studied as a function of several variables. The pH and the concentration of sodium dodecylsulphate (SDS) were found to be important. During the implementation of a central composite design for the optimization of the separation it appeared that a part of the domain was not feasible as it resulted in very long migration times and extremely deformed peaks. Therefore, a D-optimal design was selected within the boundaries of the feasible region. The optimization of the selectivity did not result in selective regions for a simultaneous separation. It was, however, possible to find a region for the simultaneous separation of 15 compounds. Further optimization at these optimal conditions resulted in a separation where 17 peaks could be observed.

Keywords: Chlorophenols; CE; Optimization; Experimental design; Irregular region; Mixed micelles

1. Introduction

Similarly to High Performance Liquid Chromatography (HPLC) [1], developing methods in Capillary Electrophoresis (CE) is a complex and difficult procedure. It is performed in several distinct steps. This paper focuses on the selection of important variables and the optimization of the selectivity. There are many variables that can affect a separation, however, optimization of the selectivity usually involves the variables related to the buffer electrolyte.

In our previous studies we focused on the separation of complex inorganic mixtures [2–4]. The migration behaviour could be described as a function of the pH and the concentration of a modifier using empirical and physical models. A similar approach is tried for organic compounds, namely a group of chlorophenols. The separation of the chlorophenol congeners has already been performed by gas [5] and liquid chromatographic [6–8] techniques. Chromatographic techniques applied for the analysis of the chlorophenol congeners usually require long analysis times. Only Ugland et al. [8] reported a separation of all the

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19 congeners, using a 30 min linear gradient, in HPLC. However, not all the peaks were baseline separated.

Some CE studies were reported in which the separation of a small number of chlorophenols was addressed [9–13]. The study of Otsuka et al. [14] is the only report that describes a separation of all the chlorophenol congeners. By applying a univariate optimization procedure with the micellar electrokinetic capillary chromatographic method (MECC), Otsuka et al. [14] achieved a separation in which not all the peaks were baseline separated. The purpose of this study, is therefore, to find the global optimum conditions for a

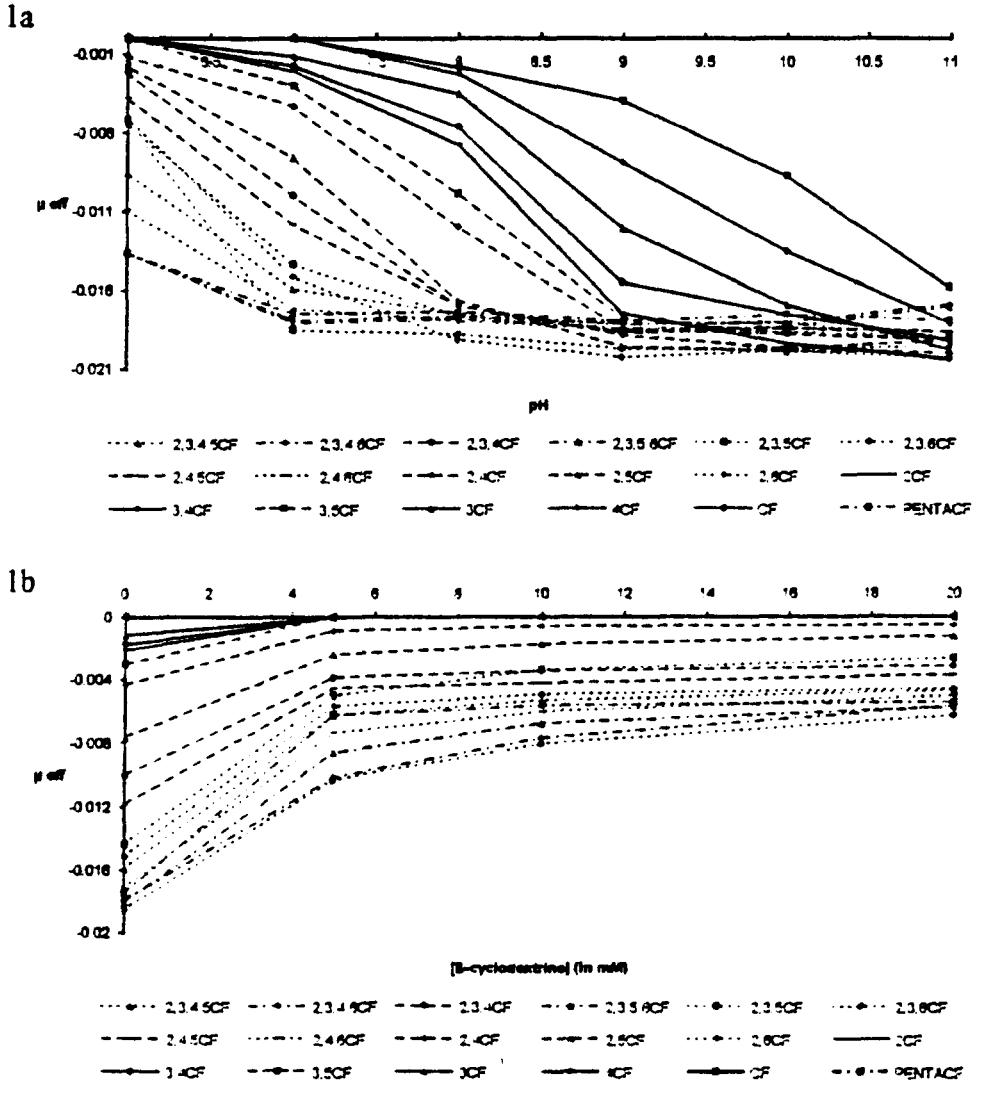

Fig. 1. The infuence of the variables on the effective mobility of the chlorophenols (CF). a, For the pH, which was varied from 6 to 11 at a pyrophosphate concentration of 10 mM. b, For β-cyclodextrine, the pH was set at 7 and the pyrophosphate concentration was 10 mM. c, For the percentage acetonitrile in the buffer electrolyte (v/v), the pH was set at 7 and the pyrophosphate concentration was 10 mM. d, For the concentration of SDS in the buffer electrolyte, the pH was set at 8 and the pyrophosphate concentration was 10 mM.

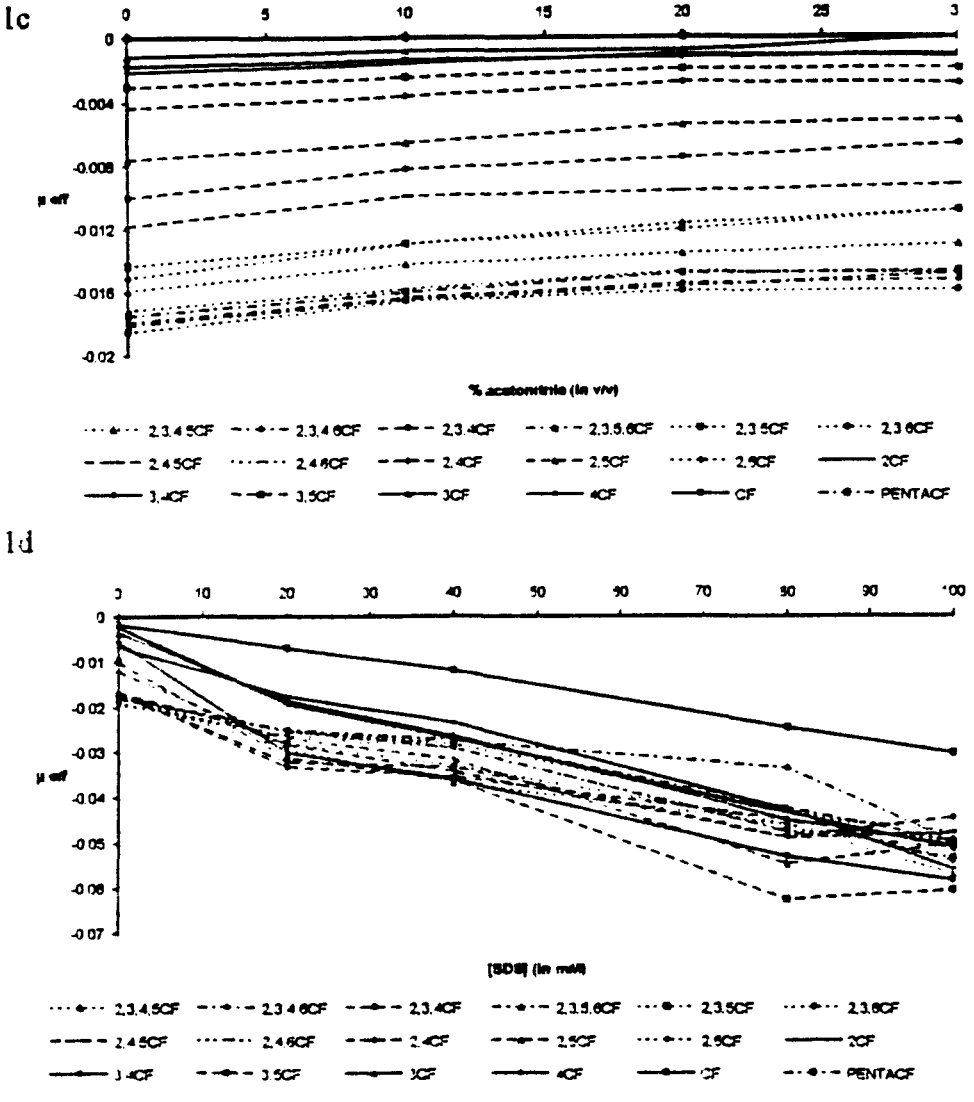

Fig. 1.

simultaneous (full) separation of 17 chlorophenol congeners and phenol (18 compounds). We aimed to achieve this by applying a simultaneous multivariate optimization strategy, instead of the univariate procedure performed by Otsuka et al. [14]. A simultaneous multivariate optimization approach implies the use of experimental statistical designs. The compounds 2,3-dichlorophenol and 3, 4, 5-trichorophenol were not included in this study because they were not available in the laboratory.

2. Experimental

2.1. Chemicals

The solutions were prepared using Milli-Q water (Millipore, Bedord, MA). All the chlorophenols were of reference grade and were obtained from Adrich. Di-sodium-diyhdrogen-pyrophosphate, tetrabutylammonium hydrogensulphate (TBAHS) and lauryl sulphobetaine (SB-12) were also ob-

tained from Aldrich. Sodium dodecylsulphate was obtained from Bios Coutelier (Belgium). Hydroxypropyl-β-cyclodextrine was obtained from Roquette (Belgium). Sodium and potassium hydroxide, hydrochloric acid, methanol, acetonitrile and urea were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of solutions

Disodium-dihydrogen-pyrophosphate was prepared as a 0.05 M stock solution. The final buffers were prepared by diluting 5 times the stock solution in 100 ml aliquots and filtering through a 0.45 μm Milex™ syringe filter (Millipore, Molsheim, France). The buffers were adjusted to the final pH using hydrochloric acid 1 M and sodium hydroxide 2 M. Stock solutions of 1000 mg l^{-1} of each chlorophenol were prepared in methanol and stored in a refrigerator. Standard solutions were prepared daily in concentration of 25 mg l^{-1}. The buffers were adjusted to the pH required using an ORION Model 520 A pH-meter.

2.3. Instrumentation

The analyses were performed on a Waters Quanta-4000 Capillary Electrophoresis system, ambient temperature controlled (20°C) and equipped with a positive power supply. All the experiments were carried out at +15 kV. The capillaries were ordinary fused-silica capillaries (Waters AccuSep™, 60 cm capillaries) with 75 μm i.d., and 52 cm length from the point of sample introduction to the point of detection. The detection was carried out by a fixed-wavelength UV-detector at 214 nm. Hydrostatic injection (25 s) was used for the injection of the samples. The electropherograms were recorded and integrated with a Waters 810 data workstation equipped with a 'W51-watchdog' interface.

2.4. Preparation of the capillary

Prior to use, a new capillary was conditioned with 0.5 M KOH for 15 min. Each time before changing a buffer the capillary was purged with 0.1 M KOH for 5 min, followed by 5 min with Milli-Q water and the buffer electrolyte. Between each run the capillary was flushed with 0.1 M NaOH for 30 s, followed by 1 min flushing with Milli-Q water and the running buffer, respectively. Before shut-down the capillary was flushed for 5 min with 0.5 M KOH and Milli-Q water, respectively. The inlet and outlet of the capillary were kept in Milli-Q water.

2.5. Calculations

Calculations were made using spreadsheets in Microsoft Excel (4.0) and Matlab (4.0) for Windows. The coefficients in the model were determined by multiple linear regression methods using the SPSS-PC + Statistics™ version 5.0 for MS-windows (3.1) software package. The D-optimal design was selected with the aid of a program written in Matlab (4.0) for windows.

3. Results and discussion

3.1. Selection of variables (1)

In the first step variables were investigated in order to study their influence on the effective mobility. These included the pH, the concentration of β-cyclodextrine (BCD), the percentage of acetonitrile and the concentration of sodium dodecyl sulphate (SDS) in the buffer electrolyte. The effective mobility (μ_{eff}, cm V^{-1} s^{-1}) was obtained by the expression

$$\mu_{eff} = \left(\frac{l*L}{V}\right)*\left(\frac{1}{T_m} - \frac{1}{T_{eof}}\right) \quad (1)$$

where l is the length of the capillary from the inlet to the point of the detection, L the total length of the capillary, V the applied voltage, T_m the migration time of a compound and T_{eof} the migration of the peak of the electroendosmotic flow (EOF) marker (methanol). The effect of pH on the effective mobility of the 18 compounds is shown in Fig. 1a. As can be observed the mobility of the

Table 1
Chlorophenols studied, classified according to their pK_a

Compound	Legend in Fig. 3, Fig. 9, Fig. 10	Abbreviation	pK_a
Group 1			**10-8**
Phenol	1	CF	9.92
4-Chlorophenol	2	4CF	9.37
3-Chlorophenol	3	3CF	8.97
3,4-di-Chlorophenol	4	34CF	8.62
2-Chlorophenol	5	2CF	8.52
3,5-di-Chlorophenol	6	35CF	8.25
Group 2 **8-6**			
2,4-di-Chlorophenol	7	24CF	7.9
2,5-di-Chlorophenol	8	25CF	7.51
2,3,4-tri-Chlorophenol	9	234CF	6.97
2,6-di-Chlorophenol	10	26CF	678
2,4,5-tri-Chlorophenol	11	245CF	6.72
2,3,5-tri-Chlorophenol	12	235CF	6.43
2,4,6-tri-Chlorophenol	13	246CF	5.99
Group 3 **6-4**			
2,3,6-tri-Chlorophenol	14	236CF	5.8
2,3,4,5-tetra-Chlorophenol	15	2345CF	5.64
2,3,4,6-tetra-Chlorophenol	16	2346CF	5.22
2,3,5,6-tetra-Chlorophenol	17	2356CF	5.03
penta-Chlorophenol	18	23456CF	4.74

2,3-di-Chlorphenol and 3,4,5-tri-Chlorophenol are not included.

compounds is highly affected by the pH. Some compounds show a sigmoid behaviour, while others show a curvilinear behaviour. The shape of the behaviour depends on the location of the pK_a of the compounds. When the pK_a (Table 1) is situated well within the experimental region, a sigmoidal shape is observed. In cases where the pH does not offer sufficient selectivity for a separation, one has to consider other selectivity adjusters. In CZE this usually means applying modifiers. Cyclodextrines are known to be interesting modifying agents, especially BCD for small organics, as they are able to retain compounds selectively in their internal cavity [15]. The inclusion of a compound in the internal cavity of the cyclodextrines depends on the dimensions and the structure of that compound. β-Cyclodextrine columns have been applied successfully for the separation of chlorophenols in HPLC [16,17]. Therefore the influence of BCD on the mobility of the compounds in this complex mixture was investigated and the results are shown in Fig. 1b. As can be observed the effective mobility of the compounds increases generally in the direction of the mobility of the EOF (the BCD's are neutral compounds that migrate with the velocity of the EOF). There are some important effects on the selectivity.

Table 2
The experimental conditions of the runs in the central composite design

Run	X_1	pH	X_2	[SDS] in mM
1	-1	7	-1	25
2	1	9	-1	25
3	1	7	1	65
4	–	9	1	65
5	$-\sqrt{2}$	6.6	0	45
6	$+\sqrt{2}$	9.4	0	45
7	0	$-\sqrt{2}$	16.7	9
0	8	$+\sqrt{2}$	73.3	9
0	8	0	45	

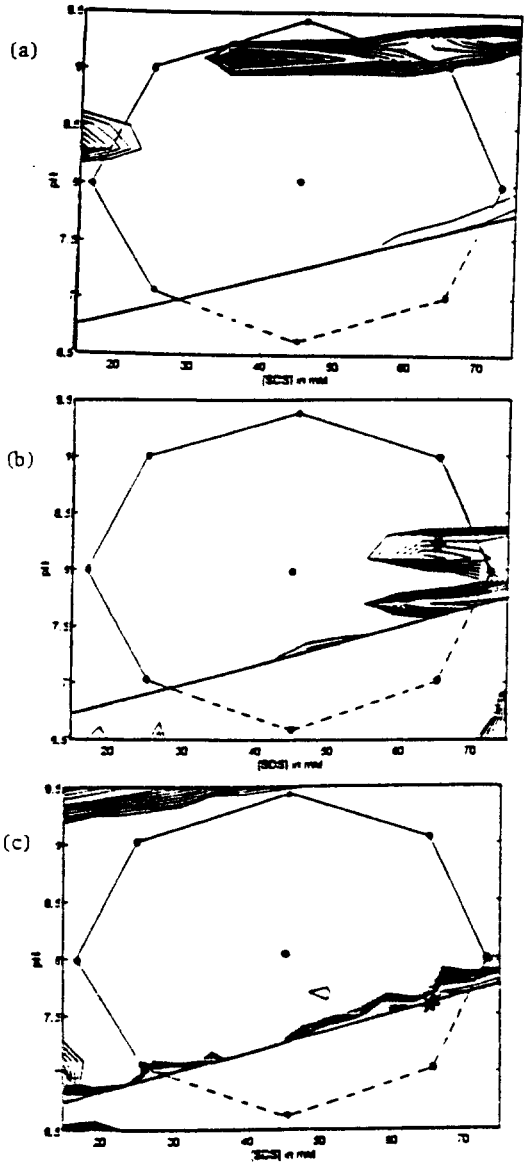

Fig. 2. Contour plots of the response surfaces for the minimum resolution as a function of the pH and [SDS] over the whole experimental domain, for group 1 (a), 2 (b) and 3 (c), respectively. The contour lines range from 1.2 to 5.0. The shape of the experimental domain which was determined by a central composite design is outlined and the part of the region that was not covered by the model calculation is shaded. Selection optial conditions are indicated in the plots by a star (*).

Organic solvents are known to have an influence on the mobility of compounds due to their influence on the viscosity of the medium and the effect on the solvation of ions. They are usually used as modifiers in a separation. The influence of acetonitrile on the mobility of the chlorophenols is shown in Fig. 1c. As can be observed, there are some minor changes in selectivities. In organic analysis by CE one often utilizes the technique of MECC. With MECC it is possible to obtain additional selectivity based on partition. The addition of a surfactant to the buffer electrolyte above its critical micellar concentration (cmc), results in the formation of micelles that induce micellar interaction based on polarity. The more apolar a compound is, the more it is attracted by the apolar inner part of the micelles. The result of the addition of SDS to the buffer electrolyte on the effective mobilities of the chlorophenols is shown in Fig. 1d. It is observed that the migration of all the compounds is reduced due to the influence of the micelles. Moreover, the selectivity is also highly affected.

3.2. Selection of an experimental design (1)

From Fig. 1 it follows that the pH and the concentration of SDS have the highest effect on the selectivity. These two variables were therefore selected for further optimization of the separation using a central composite design. The boundaries of the experimental domain were based on the preliminary experiments and the literature. According to the literature [15], the EOF has a sufficiently high intensity at conditions with pH higher than 6. Therefore the experimental domain was placed in the region where the pH is higher than 6. A pH of 8 was selected as the central point of the experimental domain as the pK_a of the compounds ranged from 5–10 (Fig. 1a). At this pH, the influence of the concentration of SDS was investigated (Fig. 1d). All the compounds resulted in acceptable migration times during these experiments. The following limits were selected for the central composite design: pH between 6.5 and 9.5 and concentration of SDS between 15 and 75 mM. The design is given in Table 2.

When performing the experiments of the design, it was noticed that in the region below the

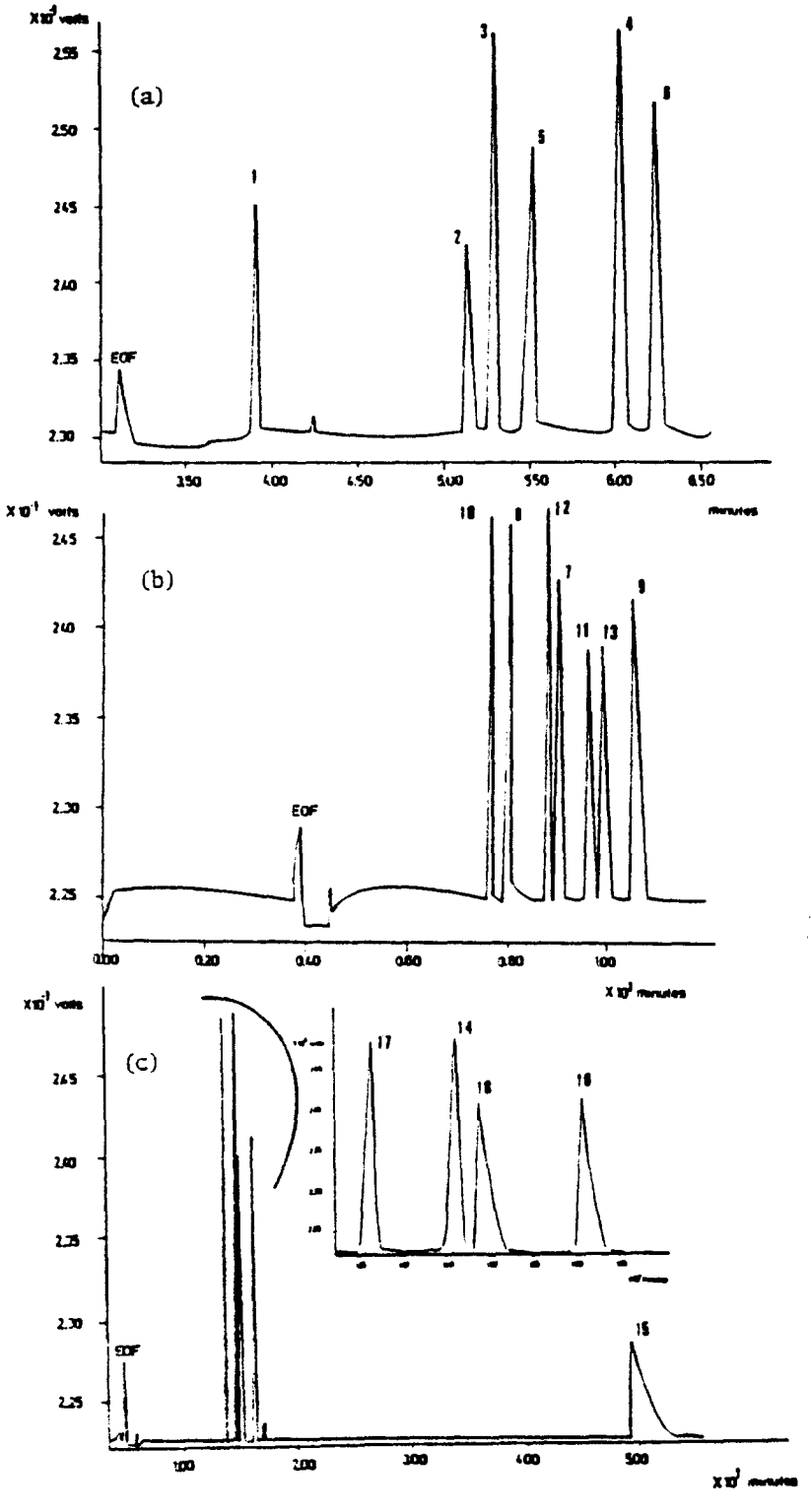

Fig. 3. Electropherograms obtained at the optimal separation conditions of each group 1 (a), 2 (b) and 3 (c), respectively. See Table 1 for the legend.

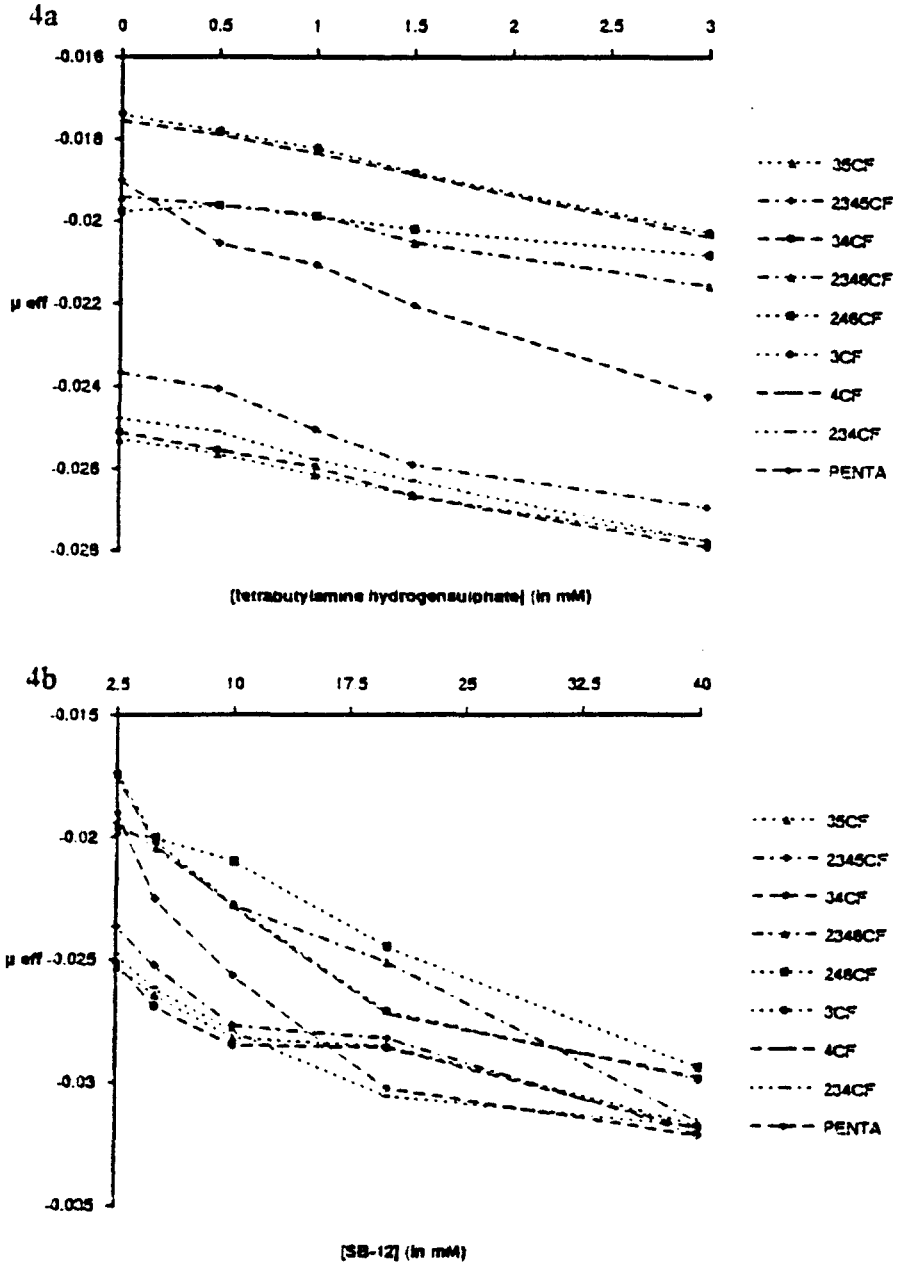

Fig. 4. Influence of the mixed micellar systems on the effective mobility of 8 selected chlorophenols. a, Shows the influence of the concentration of TBAHS, while b shows that of SB-12 in SDS containing systems. The [SDS] is equal to 40 M, pH 8.0, [pyrophosphate] = 10 mM.

line through the points pH 6.75 ([SDS] = 15 mM) and pH 7.75 ([SDS] = 75 mM) (Fig. 2), the migration times of some chlorophenols increased to unacceptable values. Moreover, the peaks were extremely deformed. The reason for this effect was found in the decreasing EOF and the increased retention of the solutes by the micelles at these conditions. Otsuka et al. [14] already de-

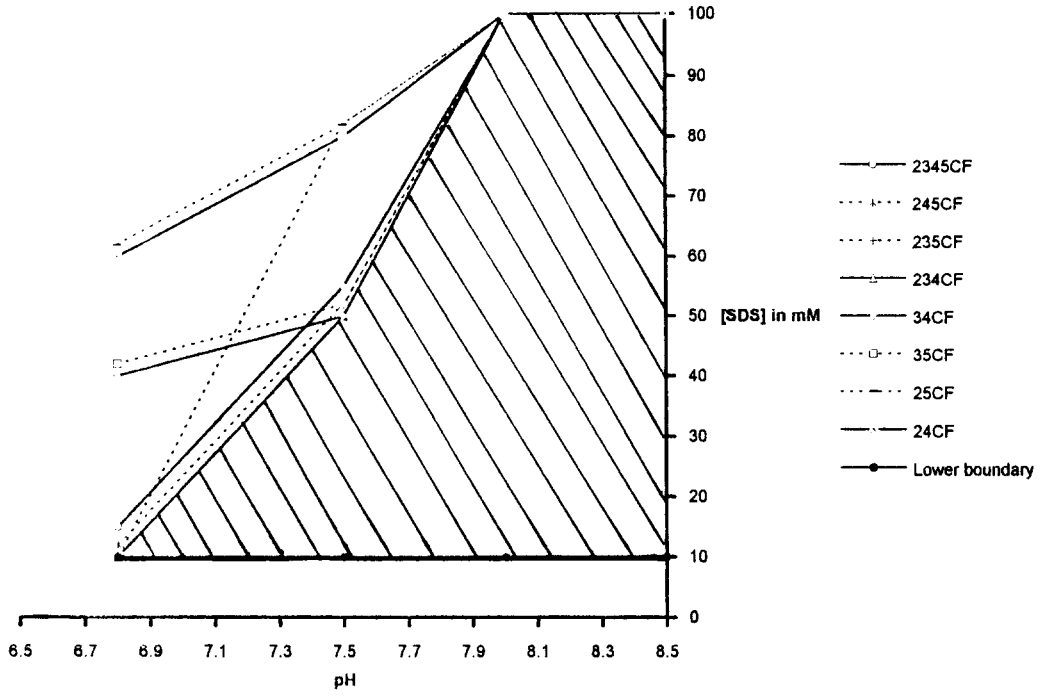

Fig. 5. The migration boundary map (MBM) obtained by examing the migration behaviour of the 8 most critical chrophenols with regard to migration. The feasible experimental region is situated below the lowest interpolation lines of the highest possible SDS concentrations and above the highest linear interpolation lines of the lowest possible SDS concentrations, indicated by the shaded area.

scribed the increased retention of the chlorophenols in this region of the domain, but it was not reported that CE of the chlorophenols in this region was unfeasible. The main difference compared to their experiments is the temperature (which was 35°C in their experiments). As the Waters CE-system is ambient temperature controlled, working at elevated temperatures was not possible.

3.3. Selectivity optimization (1)

A central composite design was first applied. Unfortunately, a part of the design was situated in the unfeasible region. These included runs 3 and 5 of the design (Table 2). The other runs of the design were performed and the optimization procedure was carried out as planned. The modelling of the electrophoretic behaviour can be performed empirically [2,4] or physically [3,4,10,11]. Empirical modelling of response like retention, resolution, migration etc. in chromatography [18,19] and CE [2,4] is usually performed with second order polynomials as the model. When the obtained response surface is sigmoid the application of higher order polynomials are more appropriate. Although a small number ($\pm$ 3) of the chlorophenols may have sigmoid surfaces, a second order polynomial was still applied. This modelling resulted in acceptable predictions of the mobilities and peak widths, allowing a selectivity optimization. The effective mobility of each substance, the mobility of the EOF and the peak widths at half height were modelled and the resolution between all the peaks in the experimental domain could be calculated. Since the separation between the two peaks is not only determined by the difference in mobility, but also by the shape and the width of the peaks, the optimization criterion: minimum absolute difference in mobility between two peaks, used in previous studies [3,4], is only applicable when the

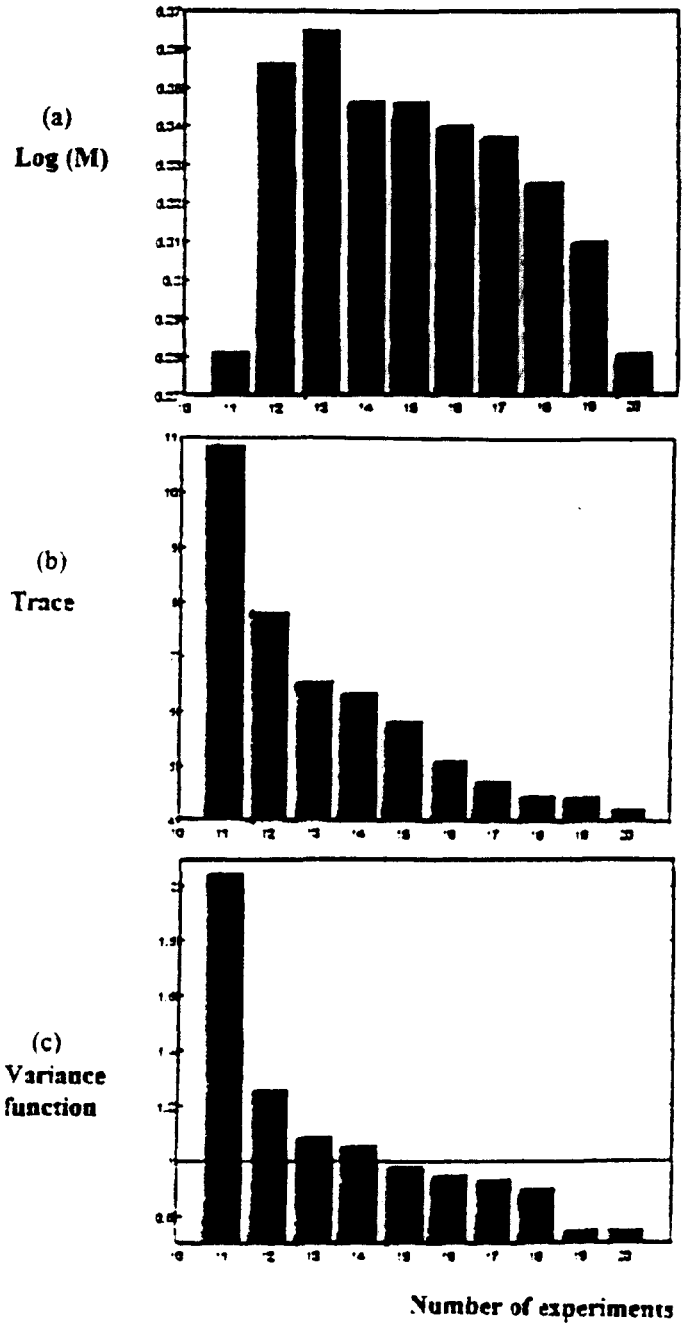

Fig. 6. Optimal criteria values for sets of experimental points (designs) with 11–20 runs. a, Is a plot of the log value of the calculation determinants (**M**) of (**X'X**)/n as a function of the number of experimental points. b, Shows the calculated values of the A-criterion or the trace (of the selected designs in Fig. 6a) as a function of the number of experiments and c the variance function.

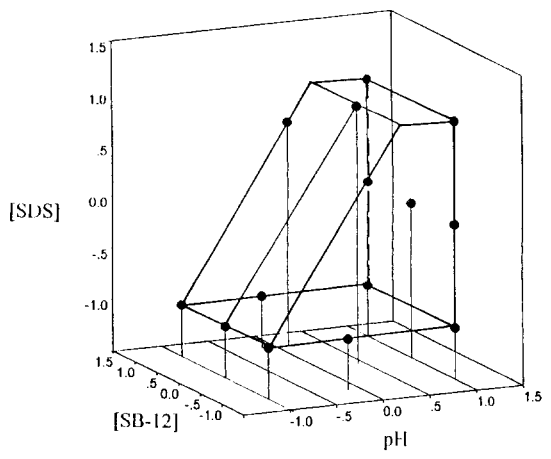

Fig. 7. The feasible experimental region with the selected experimental points of the D-optimal design.

peak widths and the shape of the peaks do not change too much as a function of the parameters. As the widths of the peaks change drastically as a function of the variables, in this part of the study, it was necessary to apply a resolution based criterion. For this reason the minimum resolution was selected as the criterion. From the predicted minimum resolution response surface including all the 18 compounds it was observed that there was no region within the experimental domain allowing separation of all the compounds simultaneously. Therefore, the chlorophenols were categorized in 3 groups according to their pK_a values (Table 1). Minimum resolution contour plots (with contour lines starting from $Rs_{min} = 1.2–5$) were generated for the 3 groups separately and are shown in Fig. 2a, b and c for group 1, 2 and 3, respectively.

For group 1 (Fig. 2a), a small selective region is observed at $9 < pH < 9.3$ and 35 mM < [SDS] < 50 mM. Separation conditions were selected in this region: pH 9.1 and [SDS] = 40 mM. This resulted in a good separation of all the peaks within a short analysis time. The electropherogram obtained for the compounds of group 1 at the selected optimal conditions is shown in Fig. 3a. The compounds of group 2 showed a selective region at high [SDS] levels and pH levels around 8 (Fig. 2b). Therefore, separation conditions were selected at pH 8.2 and [SDS] = 65 mM. The resulting electropherogram is shown in Fig. 3b. As can be seen, all the compounds are fully separated within a short analysis time. The contour plot of the minimum resolution for group 3 is presented in Fig. 2c. In the region covered by the model calculations there is a selective region close to the border at low pH and high [SDS] levels. The result of the selected conditions pH 7.5 and [SDS] = 65 mM is shown in Fig. 3c. As can be observed in the electropherogram a good separation is recorded. However, as expected the analysis time was quite long (± 50 min) and the peak shape of the last peak was strongly deformed.

Table 3
The selected D-optimal design

Run	Scaled pH	pH	Scaled [SDS]	[SDS] in mM	Scaled [SB-12]	[SB-12] in mM
1	−1	7.1	−1	10	−1	0
2	−0.14	7.7	−1	10	−1	0
3	1	8.5	−1	10	−1	0
4	−1	8.5	−1	10	0	15
5	−1	7.1	−1	10	1	30
6	−0.14	7.7	−1	10	1	30
7	1	8.5	−1	10	1	30
8	1	8.5	0	45	−1	0
9	1	8.5	0	45	0	15
10	0.14	7.9	0.67	68.3	−1	0
11	0.14	7.9	0.67	68.3	1	30
12	1	8.5	1	80	−1	0
13	0.43	8.1	1	80	0	15
14	1	8.5	1−1	80	1	30

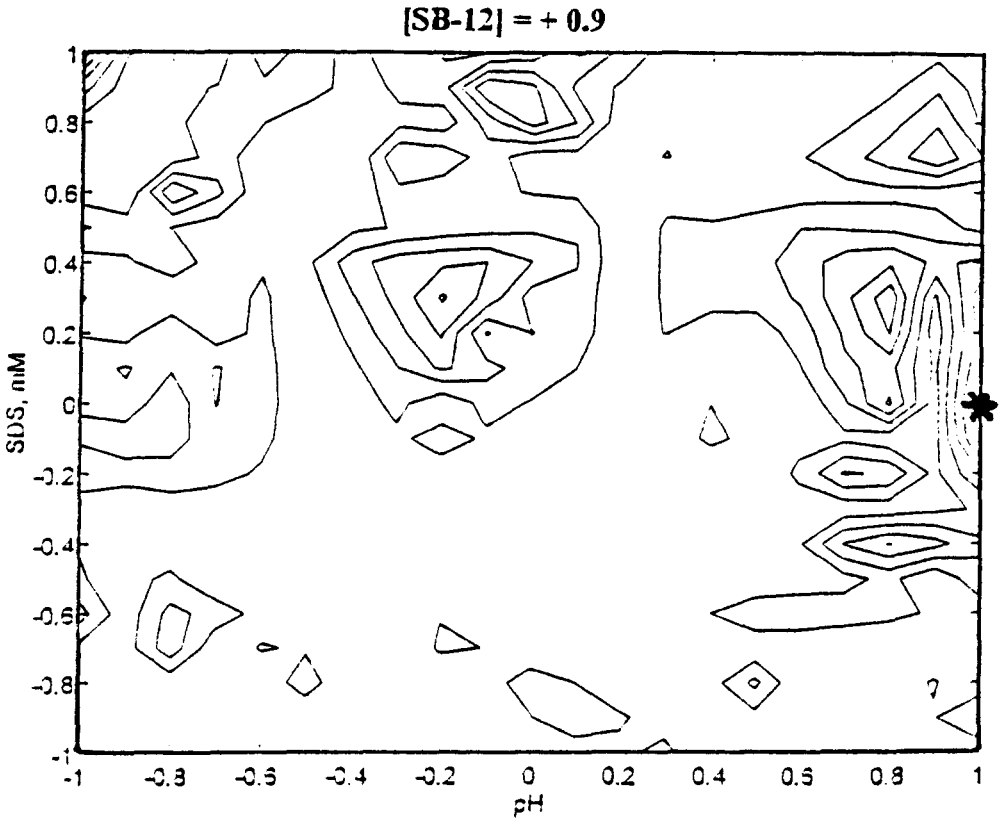

Fig. 8. The contour plot of the response surface for the minimum absolute difference in mobility of the compounds after exclusion of 246CF, 235CF and 234CF from optimization procedure. The selected optimal conditions are indicated with a star (*).

3.4. Selection of additional variables (2)

As optimization of the selectivity with the pH and the concentration of SDS as variables did not result in enough selectivity for a simultaneous separation of the 18 compounds additional variables are required for the enhancement of the selectivity. According to Ahuja et al. [20] anionic-zwitter ionic mixed micelles result in a different selectivity and a better efficiency compared to SDS alone. This was very interesting for this application and therefore two mixed micellar systems were tried out, namely SDS with tetrabutylammonium hydrogensulphate (TBAHS) and SDS with lauryl sulphobetaine (SB-12). TBAHS is a cationic surfactant and SB-12 is a zwitter ionic (neutral) surfactant, hence, these resulted in anionic–cationic and anionic–neutral mixed micellar systems. The influence of these systems was investigated with a group of 8 chlorophenols. These 8 compounds were more or less representative of the total group and could therefore be used as a subset to investigate the migration behaviour. The mixed micellar systems resulted in different selectivities as can be observed from the influence of these systems on the effective mobility of the selected compounds. Fig. 4a and b, respectively, show the effects of the concentrations of TBAHS and SB-12 on the migration of the chlorophenols in SDS containing systems. The selectivity of the two mixed micellar systems was not only different from each other, but also from the selectivity that was observed when only SDS based micelles were used. The system SDS-SB-12 was selected to be investigated further, because of the lower current production (lower Joule heating) and the good results that were obtained by Ahuja et al. [20] with this system. Finally, the pH, [SDS] and

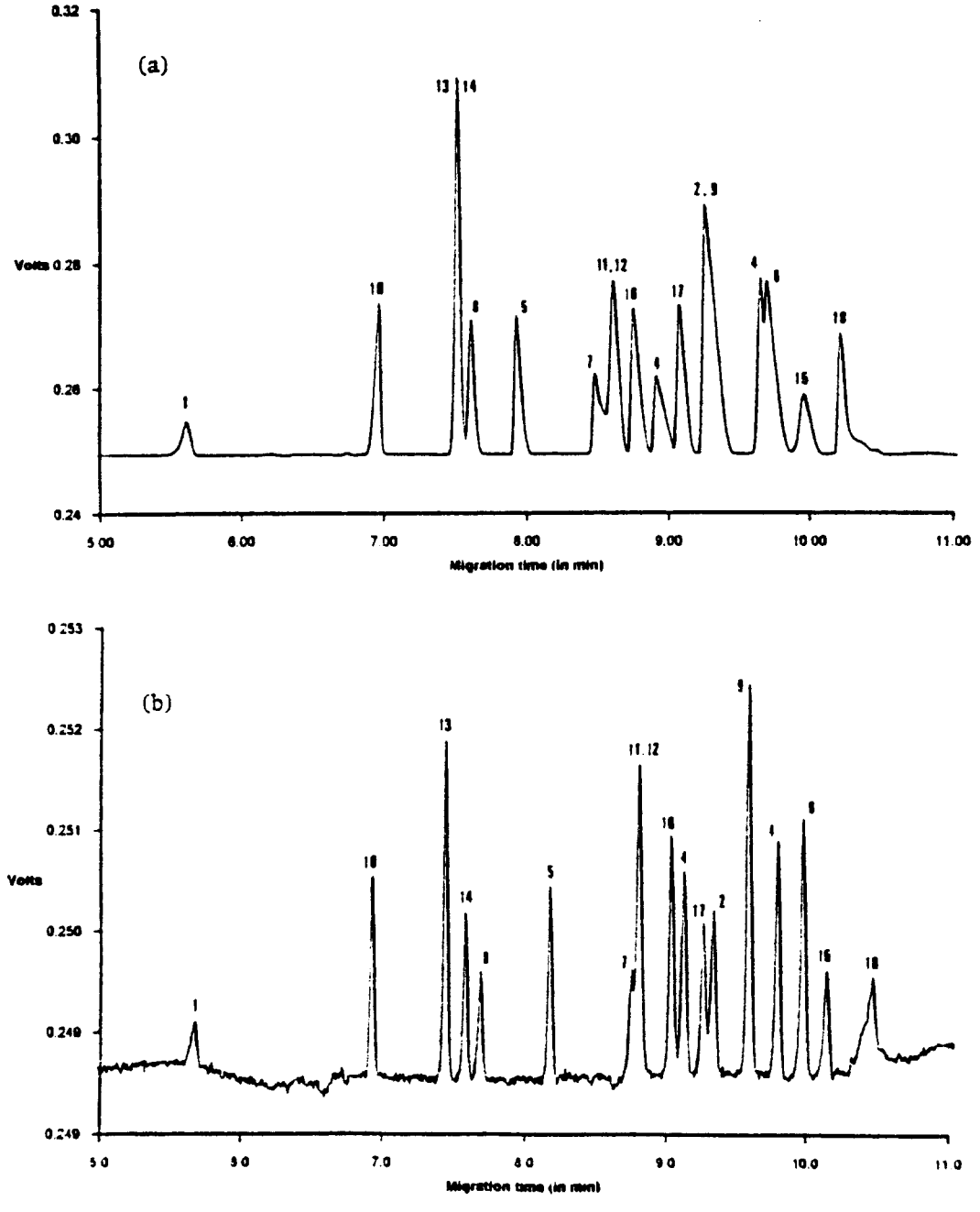

Fig. 9. Electropherograms obtained at the optimal conditions selected in Fig. 8. Fig. 9a is a separation for a sample mixture in the concentration of 25 mg l^{-1}. a, Shows the same, but for a concentration of 10 mg l^{-1}. See Table 1 for the legend.

[SB-12] were selected as important variables to optimize the separation of the 18 compounds.

3.5. Definition and outline of the feasible region

Because we are working in an irregular region, conventional classical experimental designs cannot be applied properly. In such situations it is more appropriate to apply (more advanced) flexible designs, such as D-optimal or a Kennard and Stone design [19,21,22]. D-optimal designs are based on certain mathematical criteria, whereas the Kennard and Stone design employs an algorithm that looks for the best homogeneous distribution of experimental points, based on the Euclidean distance. Prior to the definition of the design, the feasible experimental region has to be mapped exactly. For this purpose the determination of a 'migration boundary map (MBM)', similar to the retention boundary map in HPLC was applied. From preliminary experiments, it was seen that the presence of SB-12 had an influence on the selectivity, but did not affect the migration in such a way that it would influence the shape of the region. The observed irregular region was mainly due to the combination of low pH and high SDS concentrations. Therefore, the MBM was determined by plotting the highest possible concentrations of SDS that resulted in a migration time of about 25 min for each compound at the selected (low and high) pH levels. When necessary, the lowest possible SDS concentrations that result in a separation of the compounds from the neutral peak (usually this is close to the critical micellar concentration (cmc) of the surfactant) are also plotted. Next one draws linear (interpolation) lines between the SDS concentrations found at the two pH levels, for each compound. The feasible experimental region is situated below the lowest linear interpolation lines of the highest possible SDS concentrations and above the highest linear interpolation lines of the lowest possible SDS concentrations.

For the chlorophenols included in this investigation it was also known from previous experiments (Fig. 2). The region below this pH resulted in long migration times when certain amounts of SDS were added to the buffer electrolyte. Therefore, the determination of the MBM for the chlorophenols was performed by selecting 3 pH levels in the region below pH 8 (pH 7.0; 7.8 and 8.0), at which several concentrations of SDS were tried out. The [SDS] that resulted in a migration time longer than 25 min was recorded for the a group of chlorophenols. These concentrations of SDS were then plotted as a function of the pH for each of the 8 selected chlorophenols, which were most critical regarding the migration. For the points in between a linear interpolation was made. In MECC with SDS as the surfactant the lowest possible concentration is around 10 mM. The MBM obtained for the chlorophenols is shown in Fig. 5. As was expected, the acceptable migration times were obtained in an irregular experimental region (the shaded area).

3.6. Selection of an experimental design (2)

After having defined and mapped the feasible experimental region, a set of experimental points (the design) for the optimization of the selectivity and a model that is able to describe the migration behaviour were selected. As the pH in this part of the investigation ranged from pH 7.1 to 8.5, the behaviour of the compounds as a function of the pH is curved without inflection points. Therefore it can be modelled by a second order polynomial. There are three variables included in this study, resulting in the model given by the following equation.

$$Y = b_0 + b_1X_1 + b_{11}X_1^2 + b_2X_2 + b_{22}X_2^2 + B_3X_3 + B_{33}X_3^2 + B_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (2)$$

where Y is the measured response (effective mobility), X_1, X_2 and X_3 represent the variables pH, [SDS] and [SB-12], respectively and $b_0 \cdots b_{123}$ represent the multiple linear regression coefficients. Second order interaction terms are not included as they are not likely to occur.

Because the migration behaviour is modelled afterwards according to a selected model, it is preferred to select the design based on this model. This is possible with the D-optimal design, but not with the Kennard and Stone design. On the other hand, once the Kennard and Stone design is

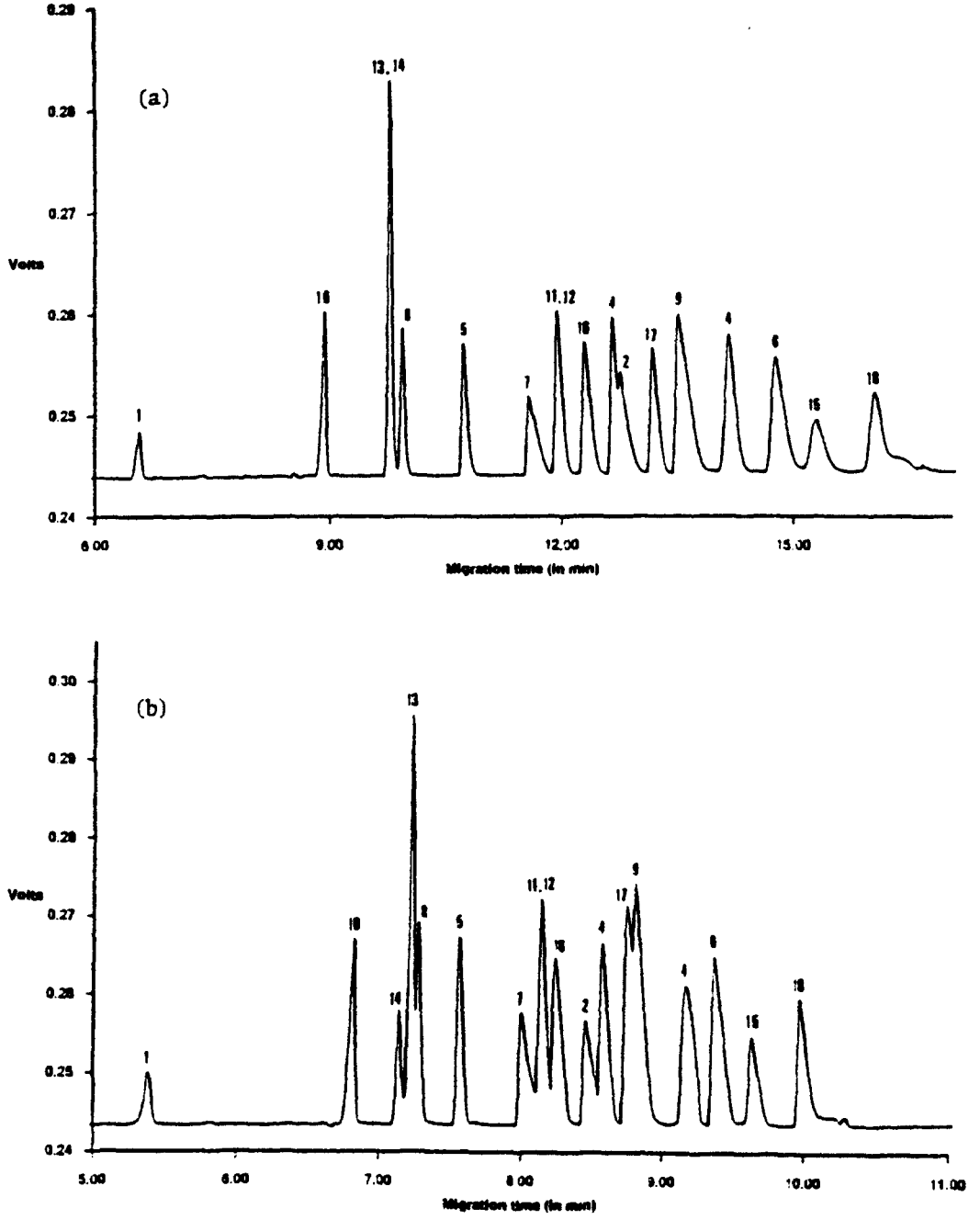

Fig. 10. The influence of the addition of urea at the optimal condition selected in Fig. 8 on the separation of the chlorophenols. a and b are separations after the addition of 1 M and 2 M urea, respectively. See Table 1 for the legend.

selected, it can be used with different models, whereas the D-optimal design is fixed to only one single model [22]. It was decided to apply the D-optimal design in this study.

The D-optimal design is based on the D-criterion. A design with n number of experiments can be considered mathematically as a matrix (**X**) of points with *n* number of rows. The D-criterion is optimal when the matrix of selected points results into a model matrix **X** with the smallest determinant of the dispersion matrix $(\mathbf{X}'\mathbf{X})^{-1}$, where **X**′ is the transposed matrix of **X**. When this is true, then the estimation of the coefficients in the model is best. The selection of a D-optimal set of experiments is based not only on the model, but also on the number of experiments. Therefore, it is performed by an iterative procedure involving the calculation of the D-criterion for selected sets of different number of points. The design with the minimum number of experimental points and where the D-criterion is optimal is selected. Sometimes there can be many different possible designs that result in an equal D-criterion making the selection of the best design difficult. In such a case one considers some additional information about these possible designs, by looking at other criteria like the A-criterion and the variance function of leverage, etc. [21,22]. The A- or trace criterion minimizes the average variance of the estimated coefficients in the model, while the variance function or leverage expresses the uncertainty in the predicted response. For more detailed information about these and other criteria one can refer to reference [21].

As there are 11 coefficients in the model, the D-criterion of designs consisting of 11–20 experiments were determined. The results are shown in the data presented in Fig. 6. Because minimising the determinant of $(\mathbf{X}'\mathbf{X})^{-1}$ is similar to maximising the determinant (**D**) of the information matrix (**X**′**X**), one therefore looks for a design with the highest **D**. According to the D-criterion, two model matrices: $\mathbf{X}_1$ and $\mathbf{X}_2$, with the same number of rows, *n* are equal when $\mathbf{DX}_1 = \mathbf{DX}_2$. In order to compare designs with different number of experiments (*n*), one defines the matrix of moments: (**X**′**X**)/*n*. The influence of the number of rows is eliminated in the determinant (**M**) of this matrix, allowing comparison of designs with different number of experiments.

Fig. 6a is a plot of the logarithmic values of the best **M**'s for a specified number of experimental points are plotted. The design with the highest **M** obtains a better D-criterion. As can be observed the designs with 12–19 experimental points result in a more or less equal D-criterion, however, the design with 13 experiments would be preferred considering this criterion. Fig. 6b and c give additional information about the designs of Fig. 6a. In Fig. 6b a plot of the A-or trace criterion is shown. The design that results in the lowest trace leads to a minimum average variance of the estimated coefficients in the model. As can be observed the trace decreases with the number of experiments. This is understandable as the more experiments one performs the more information one has about the experimental domain. Considering this criterion the design with 20 experimental points is preferred. In Fig. 6c the variance function or leverage obtained for the selected designs of Fig. 6a are plotted as a function of the number of experiments. The lower the variance function the better the accuracy of the predictions. When the variance function is equal to 1, the accuracy of the predictions is determined only by the experimental error. The design with 14 experiments is closer to this value than the design with 13 experiments. Taking into consideration all these criteria, the design with 14 experiments was selected as the D-optimal design.

3.7. Selectivity optimization (2)

The points selected by the design in the experimental region are shown in Fig. 7 and included in Table 3. These 14 runs were carried out and the effective mobilities of the compounds were calculated according to Eq. (1). The effective mobility was modelled by multiple linear regression according to the model in Eq. (2). The obtained regression coefficients of the models, the experimentally obtained effective mobilities, the relative deviations in percentages (RD%'s) between the predicted and the experimentally obtained effective mobilities, and the average relative deviations in percentage (ARD%) are shown in Table 4. As can be observed the RD%'s of runs 1 and 2 for phenol, the monochlorophenols and some of the dichlorophenols are quite high. This is probably due to the fact that these runs result in conditions

Table 4
Regression coefficients of the models, experimentally obtained mobilities and the agreement between experimental and predicted mobilities of the compounds

Regression coefficients of the models for the different compounds									
Coefficient	CF	2CF	3CF	4CF	24CF	25CF	26CF	34CF	35CF
B1	−0.01125	−0.01895	−0.02220	−0.02286	−0.02640	−0.02397	−0.02105	−0.02708	−0.02740
B1	−0.00245	−0.00670	−0.00439	−0.00366	−0.00238	−0.00159	−0.00243	−0.00379	−0.00283
B2	−0.00099	−0.00161	−0.00092	−0.00184	−0.00130	−0.00060	−0.00121	−0.00029	−0.00106
B3	−0.00764	−0.01215	−0.01035	−0.00943	−0.00683	−0.00677	−0.00490	−0.00767	−0.00643
B11	−0.00073	−0.00019	−0.00046	−0.00029	−0.00060	−0.00054	−0.00071	−0.00031	−0.00016
B22	−0.00019	−0.00041	−0.00124	−0.00138	−0.00164	−0.00060	−0.00014	−0.00238	−0.00149
B33	−0.00325	−0.00786	−0.00659	−0.00651	−0.00513	−0.00430	−0.00279	−0.00602	−0.00565
B12	−0.00193	−0.00521	−0.00414	−0.00366	−0.00260	−0.00201	−0.00283	−0.00552	−0.00398
B13	−0.00393	−0.00890	−0.00624	−0.00509	−0.00422	−0.00448	−0.00352	−0.00531	−0.00429
B23	−0.00220	−0.00601	−0.00427	−0.00330	−0.00375	−0.00427	−0.00340	−0.00529	−0.00489
B123	−0.00302	−0.00671	−0.00560	−0.00475	−0.00406	−0.00474	−0.00378	−0.00604	−0.00451
Experimentally obtained mobilities for the different compounds									
Run	CF	2CF	3CF	4CF	24CF	25CF	26CF	34CF	35CF
1	0.000	−0.001	−0.005	−0.006	−0.013	−0.015	−0.017	−0.017	−0.017
2	−0.002	−0.008	−0.008	−0.008	−0.017	−0.017	−0.019	−0.017	−0.019
3	−0.004	−0.010	−0.008	−0.007	−0.014	−0.016	−0.016	−0.013	−0.016
4	−0.012	−0.024	−0.023	−0.022	−0.025	−0.026	−0.023	−0.028	−0.028
5	−0.013	−0.019	−0.019	−0.019	−0.021	−0.021	−0.020	−0.021	−0.021
6	−0.012	−0.018	−0.019	−0.018	−0.020	−0.020	−0.020	−0.020	−0.020
7	−0.013	−0.019	−0.020	−0.020	−0.021	−0.021	−0.021	−0.020	−0.020
8	−0.008	−0.017	−0.018	−0.017	−0.021	−0.020	−0.020	−0.023	−0.023
9	−0.013	−0.022	−0.024	−0.025	−0.026	−0.023	−0.022	−0.028	−0.028
10	−0.015	−0.023	−0.026	−0.025	−0.027	−0.026	−0.021	−0.028	−0.028
11	−0.018	−0.026	−0.028	−0.028	−0.030	−0.029	−0.024	−0.031	−0.031
12	−0.011	−0.019	−0.021	−0.021	−0.023	−0.022	−0.020	−0.026	−0.026
13	−0.014	−0.023	−0.026	−0.026	−0.028	−0.026	−0.022	−0.029	−0.030
14	−0.017	−0.024	−0.027	−0.027	−0.028	−0.025	−0.023	−0.030	−0.030
Agreement between experimental and predicted mobilities (RD% and ARD%)									
Run	CF	2CF	3CF	4CF	24CF	25CF	26CF	34CF	35CF
1	−2027.8	−530.7	−38.4	−25.1	−12.9	−12.3	−7.0	−8.9	−8.4
2	19.5	19.2	9.9	10.8	8.2	5.8	5.3	4.4	3.5
3	−7.4	−5.8	−5.6	−6.7	−4.0	−0.5	−4.6	−3.3	−0.3
4	12.9	17.3	12.7	8.4	7.3	10.1	5.1	7.6	7.8
5	−4.3	−6.2	−5.3	−2.2	−0.6	−3.6	−0.1	−3.0	−3.5
6	−3.1	−8.6	−4.2	−4.6	−6.8	−5.0	−5.0	−3.8	−3.2
7	2.0	3.1	2.3	2.4	2.7	0.4	3.5	2.1	0.2
8	11.8	11.6	9.5	6.7	3.9	3.2	5.9	5.3	2.7
9	−12.3	−18.9	−12.2	−7.3	−6.8	−11.3	−5.4	−7.4	−78
10	3.7	9.3	4.8	2.6	3.6	7.7	0.0	3.2	5.5
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	−0.9	0.3	−0.6	−0.7	0.1	1.5	−1.4	−0.4	0.9
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.6	−0.3	0.4	0.4	−0.1	−1.4	1.3	0.3	−0.8
ARD%	4.9	6.8	7.6	5.6	4.1	4.5	3.2	3.5	3.2

Table 4 (continued)

Regression coefficients of the models for the different compounds										
Coeffi-cient	234CF	235CF	236CF	245CF	246CF	2345CF	2346CF	2356CF	23456CF	EOF
B1	−0.02828	−0.02490	−0.02119	−0.02746	−0.02134	−0.02577	−0.02752	−0.02133	−0.02466	−0.03274
B1	−0.00153	−0.00270	−0.00119	−0.00123	−0.00053	−0.00327	−0.00174	−0.00419	−0.00283	−0.00756
B2	−0.00240	−0.00287	−0.00031	−0.00201	−0.00054	−0.00080	−0.00308	−0.00243	−0.00009	−0.00160
B3	−0.00433	−0.00409	−0.00384	−0.00485	−0.00443	−0.00687	−0.00323	−0.00887	−0.00729	−0.00369
B11	−0.00100	−0.00351	−0.00212	−0.00073	−0.00149	−0.00056	−0.00036	−0.00062	−0.00120	−0.00164
B22	−0.00152	−0.00155	−0.00026	−0.00168	−0.00027	−0.00044	−0.00025	−0.00201	−0.00104	−0.00193
B33	−0.00388	−0.00329	−0.00155	−0.00366	−0.00175	−0.00563	−0.00510	−0.00570	−0.00589	−0.00047
B12	−0.00059	−0.00295	−0.00040	−0.00140	−0.00001	−0.00320	−0.00042	−0.00364	−0.00241	−0.00165
B13	−0.00171	−0.00289	−0.00188	−0.00266	−0.00262	−0.00484	−0.00027	−0.00628	−0.00448	−0.00370
B23	−0.00297	−0.00407	−0.00275	−0.00350	−0.00291	−0.00608	−0.00102	−0.00762	−0.00643	−0.00415
B123	−0.00303	−0.00179	−0.00260	−0.00322	−0.00214	−0.00495	−0.00099	−0.00598	−0.00468	−0.00492
Experimentally obtained mobilities for the different compounds										
Run	234CF	235CF	236CF	245CF	246CF	2345CF	2346CF	2356CF	23456CF	EOF
1	−0.022	−0.022	−0.022	−0.021	−0.019	−0.019	−0.019	−0.018	−0.020	−0.035
2	−0.021	−0.020	−0.019	−0.021	−0.019	−0.021	−0.018	−0.018	−0.018	−0.034
3	−0.016	−0.026	−0.018	−0.016	−0.019	−0.020	−0.014	−0.020	−0.021	−0.042
4	−0.029	−0.028	−0.025	−0.028	−0.024	−0.028	−0.027	−0.027	−0.028	−0.031
5	−0.024	−0.024	−0.024	−0.024	−0.023	−0.021	−0.021	−0.020	−0.020	−0.029
6	−0.02	−0.020	−0.020	−0.020	−0.020	−0.021	−0.021	−0.021	−0.022	−0.036
7	−0.022	−0.022	−0.022	−0.020	−0.021	−0.022	−0.022	−0.022	−0.022	−0.044
8	−0.021	−0.020	−0.019	−0.021	−0.019	−0.021	−0.018	−0.018	−0.020	−0.044
9	−0.027	−0.024	−0.022	−0.026	−0.022	−0.026	−0.024	−0.024	−0.028	−0.042
10	−0.028	−0.026	−0.023	−0.027	−0.023	−0.027	−0.024	−0.024	−0.027	−0.039
11	−0.031	−0.030	−0.025	−0.031	−0.026	−0.030	−0.028	−0.028	−0.030	−0.039
12	−0.023	−0.021	−0.019	−0.023	−0.019	−0.023	−0.019	−0.019	−0.022	−0.043
13	−0.029	−0.028	−0.022	−0.028	−0.022	−0.028	−0.030	−0.024	−0.028	−0.037
14	−0.028	−0.026	−0.024	−0.027	−0.024	−0.028	−0.027	−0.027	−0.030	−0.044
Agreement between experimental and predicted mobilitie s (RD% and ARD%)										
Run	234CF	235CF	236CF	245CF	246CF	2345CF	2346CF	2356CF	23456CF	EOF
1	−6.9	−3.9	−2.3	−6.5	−4.1	−7.8	−5.9	−4.3	−0.3	−3.9
2	7.5	0.3	2.2	6.6	3.7	3.6	2.6	−1.5	−4.5	−5.7
3	−2.5	3.5	−0.7	−0.6	−0.6	1.0	−2.9	1.8	3.9	−0.9
4	4.2	5.7	2.1	4.4	3.0	7.1	6.1	6.7	3.7	−1.6
5	1.3	−3.2	−0.1	0.6	0.1	−2.7	−2.7	−5.3	−4.9	−2.9
6	−7.8	−0.3	−2.1	−6.6	−3.4	−3.6	−2.2	1.3	3.8	5.3
7	1.8	−4.2	0.5	0.5	0.5	−0.9	1.9	−1.7	−3.8	0.9
8	0.2	−5.3	0.8	−1.6	0.0	−0.2	6.9	2.3	−2.2	4.9
9	−4.6	−6.7	−2.5	−4.8	−3.3	−7.6	−6.9	−7.6	−3.8	−1.2
10	4.2	10.2	1.7	5.8	3.1	7.7	1.7	5.8	5.4	−6.9
11	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0
12	−1.2	−4.4	−0.3	−2.1	−0.9	−2.3	−1.1	−1.3	−2.2	−2.8
13	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0	−0.0
14	−1.0	−3.6	−0.2	−1.8	−0.8	−1.9	0.8	−0.9	−1.6	2.7
ARD%	3.1	3.7	1.1	3.0	1.7	3.3	3.0	2.9	2.9	2.8

were peaks of the compounds are merely separated from the EOF peak, making the determination of the effective mobilities inaccurate. For this reason these runs were not included in the modelling of the effective mobility for these compounds. Nevertheless, the average relative deviations in percentage (ARD%) between the predicted and the experimentally obtained effective mobilities were within the expected ranges, indicating acceptable predictions and thus giving a possibility for predicting the optimal conditions. The optimization criterion in this case was the minimum absolute difference in mobility between 18 compounds, because the peak widths and the migration times did not change drastically in the feasible region.

Unfortunately, once again no selective region could be found for the 18 compounds in the selected experimental domain. Therefore it was decided to search for the best possible separation that could be obtained. During the optimization procedure it was noticed that the separation of the peak pairs 236CF-246CF, 245CF-235CF and 4CF-234CF were the limiting factor for a simultaneous separation. By excluding 246CF, 235CF and 234CF from the optimization procedure, a response surface was obtained of which the contour plot is shown in Fig. 8. The selected conditions are indicated with a star (*) and are situated at the highest level of pH (+ 1: pH 8.5), nominal level of [SDS] (0:[SDS] = 45 mM) and also at high level of SB-12. The contour plot was generated at a [SB-12] equal to + 0.9: 28.5 mM. The experimentally obtained electropherogram is shown in Fig. 9a and resulted in a separation in which 15 peaks were observed. When the sample mixture was diluted to 10 mg l^{-1} instead of 25 mg l^{-1}, a separation of most of the peaks could be achieved. The peak pair 245CF-235CF was still not separated (Fig. 9b).

Following this, we investigated whether the addition of a fourth parameter to the determined optimal conditions, would result in a full separation of all the peaks. For this reason, different amounts of urea were added to the optimal conditions obtained from the simultaneous optimization step. Urea is known to expand the separation window in MECC [15], which can result in a better separation. The results of the addition of 1 M and 2 M urea in the buffer electrolyte are observed in Fig. 10a and b, respectively. As can be seen in Fig. 10a, the addition of 1 M urea resulted in a separation in which 16 peaks are found. The peak pairs 236CF-246CF and 245CF-235CF were still not separated. The addition of 2 M urea resulted in the separation of the peak pair 236CF-246CF, but the peak pair 245CF-235CF was still not separated (Fig. 10b). However, a separation of 17 peaks was achieved.

4. Conclusions

During the method development process many difficulties are encountered. A serious problem is the irregularity of the feasible experimental region. To our knowledge such a problem has not been reported before in CE methods development. The approach applied in chromatography for optimization in irregularly shaped experimental regions [19] appears to be applicable to CE problems after minor adjustments. For the separation of the chlorophenols, the optimization of the selectivity has to be continued by including other combinations of the variables, such as pH, [SDS] and [TBAHS] or pH, [SDS] and [urea].

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