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MULTIFACTOR SIMULTANEOUS STATISTICAL OPTIMIZATION OF THE MOBILE PHASE COMPOSITION FOR THE SEPARATION OF INORGANIC ANIONS IN REVERSED-PHASE ION-INTERACTION CHROMATOGRAPHY

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SUMMARY

The optimization of the analysis of a mixture of five anions with reversed-phase ion interaction chromatography has been investigated. The criteria selected were the resolution of the most critical peak pair, R_{s1} (NO_2^- - Br^-), the total analysis time which corresponds to a certain range of R_{s2} (NO_3^- - SO_4^{2-}) and the peak height of Cl^- , NO_3^- and SO_4^{2-} . The factors selected to realize the optimization were the mobile phase concentrations of tetrabutylammonium iodide and phthalate buffer, and the pH. Only a limited number of experimental data points, ordered in a central composite design, were required for the computation of the response function with a non-linear regression model. Application of this computer-aided multifactor simultaneous statistical approach resulted in a rapid optimization of the selected criteria. The computed global optimum is close to the best experimentally obtained result.

INTRODUCTION

Several approaches based on reversed-phase chromatography have been used, as an alternative to ion chromatography, to separate the common inorganic anions^{1–3}. Among these, applications of ion-pair chromatography (IPC) or ion-interaction chromatography (IIC) using an octadecyl-bonded silica as a non-polar stationary phase have recently been reported^{4–7}. The success of this inorganic separation technique is primarily because of the large number of variable factors that can be adjusted in the mobile phase to give improved chromatographic performance. The pH, ionic strength, the counter-ion concentration and the concentration of the hydrophobic ion-interaction or ion-pair reagent of the eluent are frequently used factors in mobile phase optimization studies^{8–11}. The theoretical dependency of the capacity factor on these factors has been studied by various authors^{6,12}. More detailed studies

on the influence of various parameters on the retention behaviour have been performed by Bartha and Vigh^{13,14}. Such studies provide the key to systematic optimization of IIC.

Papers concerning mobile phase optimization, an important topic for chromatographers can frequently be found in the recent literature. The aim of optimization is to achieve the maximum attainable separation of all components of a complex multi-component mixture of solutes within a minimum analysis time, and also, if possible, with the highest sensitivity for all solutes. In general, the optimization methods are based on either heavily modelled formalisms or purely empirical functions; the former are becoming cumbersome (except for fairly simple systems) as the number of parameters increases and the model grows more complex. When developing a chromatographic method, the existence of the multifactors with multicriteria to be optimized in order to achieve the best separation of a mixed sample complicates the optimization process. In addition, as discussed by some authors^{9,10}, optimization of chromatographic performance is especially difficult not only because of the variation in chromatographic behaviour due to the different properties of the reversed-phase materials but also because of the existence of multiple optima over the domain of mobile phase composition.

Nevertheless, many successful optimization schemes have been proposed in recent publications, such as the solvent selectivity triangle approach¹⁵, the sequential simplex technique and the chromatographic optimization function^{16,17}, the overlapping resolution mapping (ORM) method¹⁸, the "window diagram" graphic method^{9,19} and recently computer-aided procedures^{11,19-22}. Among these, the statistical approaches for optimization of discrete variables such as the pH and other ionic effects using a factorial design have been shown to be quite useful in specific analyses^{10,16,22}. Sachok *et al.*⁹ optimized the separation of a nine-component mixture of weak organic acids, bases and zwitterionic compounds using a four-level two-factor (4^2) experimental design to specify sixteen mobile phase compositions. A similar experimental design has been used by Lindberg *et al.*¹⁰ to separate four alkaloids.

In this study, a three-factor central composite design was used for the optimization experiments. We will discuss the computer-aided multifactor statistical optimization approach and the application of a polynomial mathematical model with regard to the separation of a mixture of five inorganic anions with a reversed-phase IIC system. Computer predicted response surfaces were used to describe the combined effect of three variables or factors (ion-interaction reagent concentrations, pH, buffer concentration) on the resolutions, (R_s), of some critical peaks, and their sensitivities (peak height, h), which were the criteria selected for optimization.

EXPERIMENTAL

Apparatus and reagents

The chromatographic system consisted of a DuPont 870 HPLC pump, a Valco injection valve with 100- or 50- μ l loop volume, a Partisil 10 ODS-3 RP column (250 mm \times 4.6 mm I.D., Whatman) acting as a separation column and protected with a 60 mm \times 2.1 mm guard column using the same packing, a Perkin-Elmer LC-21 conductivity detector and an Omniscrite B5 217-5 strip chart recorder. The separation column, guard column and detector cell were thermally isolated in a wooden box to

minimize short-term temperature variations. All separations were carried out at room temperature and a mobile phase flow-rate of 2 ml/min.

All water used was deionized and passed through a Millipore (Bedford, MA, U.S.A.) Milli-Q water purification system. Tetrabutylammonium iodide (TBAI, 98% for synthesis) was obtained from Merck, potassium hydrogenphthalate (KHP, A.R.) from R.C.B. (Belgium); all other reagents were of A.R. grade. Standard solutions (1000 ppm) of inorganic anions (chloride, nitrite, bromide, nitrate, sulphate and dichromate) were prepared by dissolving appropriate amounts of the corresponding potassium salts in pure water. These solutions were diluted daily to give the trace solutions required.

Mobile phases

The eluents used contained TBAI with KHP at pH between 4 and 6.5. The concentrations of TBAI ranged from 0.5 to 16 mM, and of KHP ranged from 0.5 to 2 mM. The pH of all mobile phases was adjusted by adding to a solution, containing a weighed amount of the salt, potassium hydroxide or acetic acid followed by a dilution to volume. These eluents were prepared daily, filtered through a 0.45- μ m porosity membrane filter (Millipore HSWP 04700) and degassed before use.

Column preparation

The columns were packed in the laboratory by a slurry technique using 2-propanol as a suspending medium under a pressure of 6000–6500 p.s.i. They were washed successively with 100 ml of methanol and 60 ml water before use. Column testing was carried out with our operative mobile phase. A sample containing a mixture of chloride, nitrite, bromide, nitrate and sulphate may show capacity factors, k' , between 1.3 and 7. Only columns that gave theoretical plate heights of more than 15000 were accepted for use.

METHODS

The mobile phase optimization method we applied can be considered as a stochastic statistical procedure. The essential steps in this approach are similar to those described by Snee^{2,3} in his optimization: (1) generation of data using a pre-planned experimental design; (2) finding a mathematical model to fit these data using statistical curve-fitting techniques and (3) examination of the response surfaces to determine the best value. This approach requires only a limited predefined number of experimental chromatograms which can be used for the construction of a model of the chromatographic system in question. The model can, in principle, accurately predict the behaviour of all solutes within the mobile phase envelope defined, without recourse to further experiments. In addition, optimum separation conditions can be inferred from the model. Since a critical problem is the fact that up to date no mathematical model exists which is able to predict the retention of the analytes and hence to describe the true response surface on the basis of solely fundamental principles, (1) the velocity field (advection and diffusion) in the separation column and (2) the binding kinetics of anions on the stationary phase, statistical optimization methods have to be used. These models are stochastic because of random uncertainties in the experimentally obtained data. For the same reason, these models cannot be

extrapolated to conditions which are beyond the scope of the experiments included in the model.

In order to improve the accuracy of the predictions achievable with the statistical model, a two level approach is adopted: (a) modification of the polynomial equations [for example instead of $y = f(x)$ ones uses $\log y = f(x)$] until a good agreement between experimental and calculated data is obtained and (b) verification of the predicted optimum by some new experiments which are planned around the location of the predicted optimum and/or extension of the variable values beyond the original domain of the factor design. The resulting data are used to improve the model and to calculate a new optimum.

Three-factor central composite experimental design

In order to investigate the effect of each variable on the separation and detection quality and their possible interaction, a three-factor central composite design which requires fifteen experiments according to Massart *et al.*²⁴ has been adopted as the optimization strategy. In these methods, one tries to describe the response surface in the region where the optimum is to be found using a mathematical equation. In most instances generalized polynomials are employed

$$y = b_0 + b_1x_1 + \dots + b_nx_n + b_{11}x_1^2 + b_{12}x_1x_2 + \dots + b_{nn}x_n^2 + \dots \quad (1)$$

where y is the approximate response, x_1, \dots, x_n are the factors and b_0, \dots, b_{nn} are the coefficients estimating the true and unknown coefficients $\beta_0, \dots, \beta_{nn}$. Usually, only first- or second-degree polynomials are employed, but we used a more elaborate one.

The coefficients b_0, \dots, b_{nn} are determined from a number of independent equations at least equal to the number of coefficients. If this number is m , this means that m experimental responses y_i must be determined. These are several advantages of organizing the experiments in a factorial design²⁵: (i) when there are no interactions the factorial design gives the maximum efficiency in the estimation of the effects; (ii) when interactions exist, their nature being unknown, a factorial design is necessary to avoid misleading conclusions; (iii) in a factorial design the effect of a factor is estimated at several levels of the other factors, and the conclusions hold over a wide range of conditions. In particular, it is preferable to use orthogonal factorial plans. First, the factors, x_i , which have an important effect on the response have to be defined. As we mentioned earlier, the optimization of the separation of anions involves the optimization of three factors: the mobile phase buffer concentration, pH and ion-interaction reagent concentration. Then, it is convenient to scale the factors and to express the factor levels in scaled units. As a result the base point, y_0 , of a factorial experiment has a scaled value of zero for each factor, and the other points can have values of $+1, 0, -1$. Next, one has to decide the kind of polynomial to be used to approximate the response surface, because it determines the number of coefficients, b_i , which have to be assessed. In our case, this number is eleven. We tested several polynomials (see below) and found that the best results were obtained with the usual second-order polynomial, see eqn. 1, extended with an eleventh term: $a_{10}x_1x_2x_3$ (see below). We can now carry out a one-, two-, three- or still higher-level factorial experiment. A three-factor, two-level design involves 8 experiments. This is not sufficient to determine the eleven coefficients, b_i , of the response surface polynomial.

A three-level factorial design involves 27 experiments which largely exceeds the requirements. A more efficient procedure is the use of a central composite design. $2n + 1$ (n being the number of factors) experiments are added to the 2^n design (two-level design), one of the additional experiments being performed at the base point and the others along the coordinate axes at a distance " a ". The value of " a " making a three-factor composite design orthogonal is 1.215 (ref. 25). The resulting arrangement of experimental points (15) is shown in Fig. 1.

RESULTS AND DISCUSSION

Once the experimental design has been chosen (in our case, see discussion above, a central composite design including 15 points, which include the corners of a cube, the centre point and 6 additional points), a number of decisions have to be made in an hierarchical way before model fitting and prediction can be performed.

(1) The choice of the optimization criteria. Each optimization criterion is mathematically expressed as a response function, y , of a number of factors, x_i (see eqn. 1): y is a directly measurable parameter, for example the peak height of a given anion, or can be calculated, for example the resolution of a peak pair of anions.

The choice of the optimization criteria cannot be made on a purely theoretical basis. Some preparative experiments must indicate which anions are the most difficult to separate, which anions shows the lowest detection sensitivity and which anions determine the analysis time. In a previous work, we studied yet the influence of the mobile phase composition on the chromatographic behaviour of each anion in a mixed sample based on one factor⁷ or two factors²⁶ at a time. We found that with most mobile phase compositions the peaks of NO_2^- and Br^- were only poorly separated, and showed the worst resolution of all peak pairs. The optimization of $R_s(\text{NO}_2^- - \text{Br}^-)$ becomes therefore the key criterion of the global optimization. A second important criterion regarding chemical analyses is the total analysis time. Since the total elution time is related to the resolution of the last two peaks eluted (NO_3^- and SO_4^{2-} , see Fig. 2), $R_s(\text{NO}_3^- - \text{SO}_4^{2-})$ is used to optimize the total elution time indirectly. A third criterion of analytical interest is the sensitivity of the method. Based on these considerations, we choose in order of priority the following optimization criteria: (1) the best resolution of the most critical peak pair ($\text{NO}_2^- - \text{Br}^-$), (2) an optimum

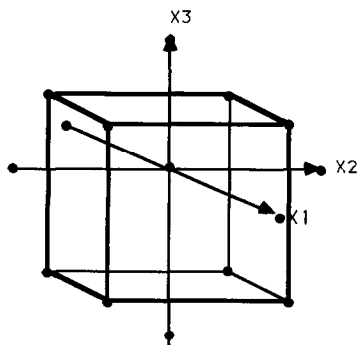


Fig. 1. Schematic representation of the three-factor central composite design.

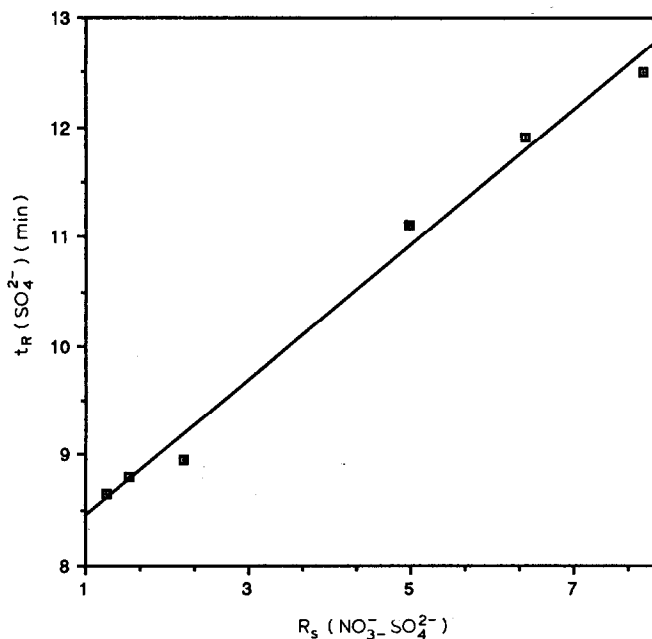


Fig. 2. Retention time dependency of the last anion peak (SO_4^{2-}) eluted from the separation column on the resolution of $\text{NO}_3^- \text{SO}_4^{2-}$. $[\text{TBAI}] = 8 \text{ mM}$, $[\text{KHP}] = 1 \text{ mM}$, pH 5.

resolution range for the peak pair $\text{NO}_3^- \text{SO}_4^{2-}$, with an under and upper limit, and (3) the best possible detection responses (peak heights in our experiments but integrated peak areas may also be used) of chloride, nitrate and sulphate.

It is clear that for other chromatographic systems, other optimization criteria and orders of priority may be chosen. For example, optimization criteria such as the "Chromatographic Response Function"^{17,27}, the "Relative Resolution Products"^{15,28} or the "Chromatographic Optimization Function"¹⁵ have been reported for the optimization of organic separation with mixed design. In this work, the realistic optimization criteria which can be selected based on our chromatographic experience are as follows: (a) the least acceptable resolution of the peak pair $\text{NO}_2^- \text{Br}^-$, R_{s1} , is 1.35; (b) the resolution of the peak pair $\text{NO}_3^- \text{SO}_4^{2-}$, R_{s2} , is restricted to the range of 1.5 to 1.8, which means that the total separation time should be less than 10 min; (c) maximum peak heights, h_i , for Cl^- , NO_3^- and SO_4^{2-} . Here no special need exists to put a restriction on h . However, if in addition to an optimum value of R_{s1} (R_{s1} exceeds 1.35) and minimum analysis time ($1.5 < R_{s2} < 1.8$) the h values exceed a given standard height, h_x , then a global optimum is found.

(2) The choice of the factors (the dependent variables x_i in eqn. 1) which will be used to optimize the selected criteria. These factors should have a major influence on the selected criterion. In a previous paper we showed that the mobile phase pH, the TBA or ion interaction reagent concentration and the buffer concentration are the variables with the largest influence on the capacity factors as well as the resolution and peak heights.

Figs. 3 and 4 which result from the data of ref. 7 show the dependency of the

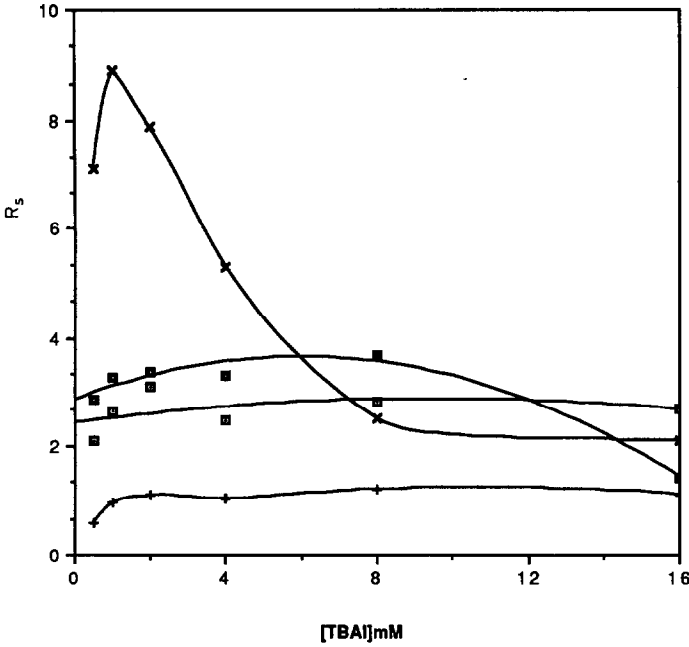


Fig. 3. Dependency of the resolution factor for several anion peak pairs on the TBAI concentration in the neat aqueous phthalate (1 mM) eluent (pH 5). Chromatographic conditions: flow-rate, 2 ml/min; Partisil 10 ODS-3 column; conductivity detection. (□) $R_s(\text{Cl}^- - \text{NO}_2^-)$; (+) $R_{s1}(\text{NO}_2^- - \text{Br}^-)$; (■) $R_s(\text{Br}^- - \text{NO}_3^-)$; (×) $R_{s2}(\text{NO}_3^- - \text{SO}_4^{2-})$.

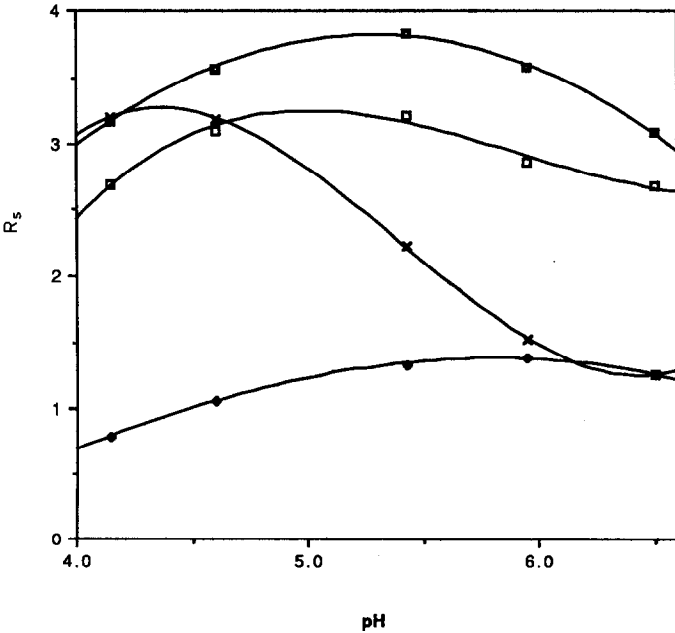


Fig. 4. Dependency of the resolution factor for several anion peak pairs on the pH with 1 mM KHP as the eluent. TBAI concentration: 8 mM. Chromatographic conditions as in Fig. 3. (□) $R_s(\text{Cl}^- - \text{NO}_2^-)$; (◇) $R_{s1}(\text{NO}_2^- - \text{Br}^-)$; (■) $R_s(\text{Br}^- - \text{NO}_3^-)$; (×) $R_{s2}(\text{NO}_3^- - \text{SO}_4^{2-})$.

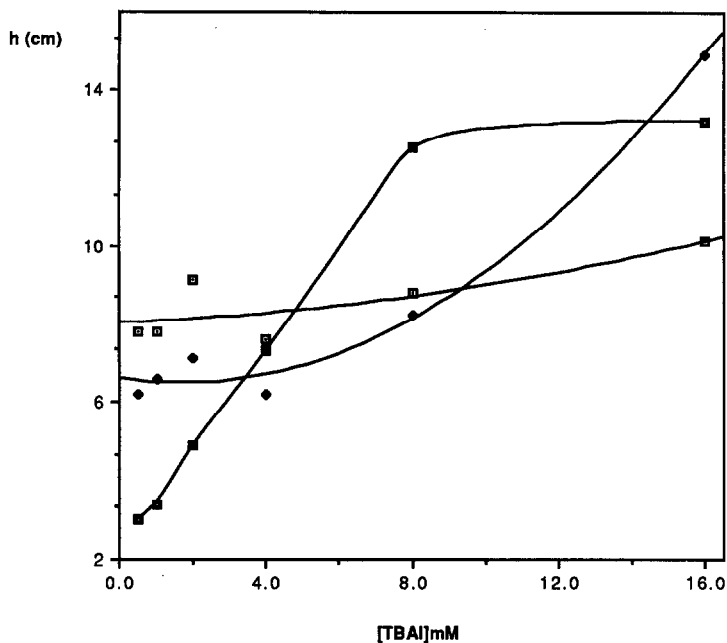


Fig. 5. Dependency of the peak height of the detected anions on the TBAI concentration in the eluent. Eluent: 1 mM KHP; pH 5. Chromatographic conditions as in Fig. 3. (□) R₁, Cl⁻; (◆) R₂, NO₃⁻; (■) R₃, SO₄²⁻.

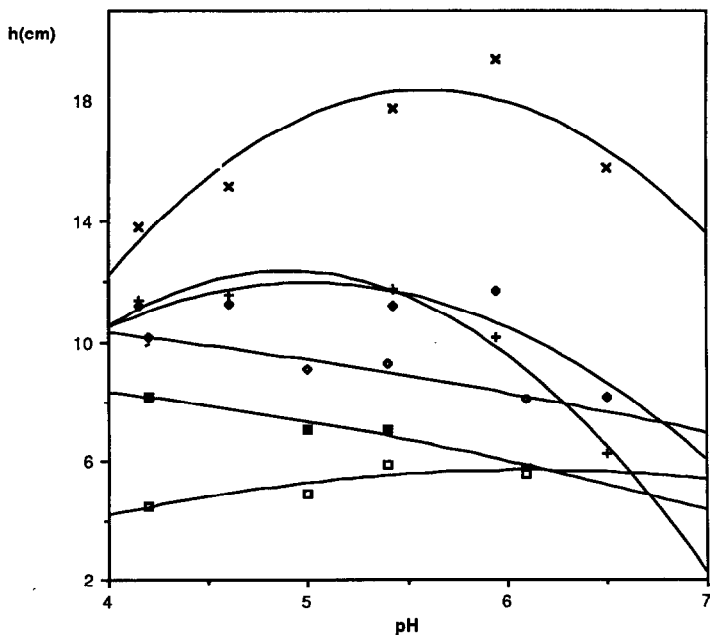


Fig. 6. Dependency of the peak height of some anions on the eluent pH. Eluent: 1 mM KHP; TBAI concentrations, 8 and 2 mM. Chromatographic conditions as in Fig. 3. (+) Cl⁻, [TBAI] 8 mM; (◆) NO₃⁻, [TBAI] 8 mM; (x) SO₄²⁻, [TBAI] 8 mM; (◇) Cl⁻, [TBAI] 2 mM; (■) NO₃⁻, [TBAI] 2 mM; (□) SO₄²⁻, [TBAI] 2 mM.

resolution, R_s , on two of the three factors chosen: [TBAI] and pH. Figs. 5 and 6 show the dependency of the peak height on [TBAI] and pH. Fig. 7 is an example of a visualized three-dimensional response surface plot²⁶ describing the dependency of the resolution of peak pair NO_2^- - Br^- on two variables [TBAI] and pH).

The criteria or response functions R_s and h can be expressed as a polynomial development of the three factors chosen

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1^2 + a_5x_2^2 + a_6x_3^2 + a_7x_1x_2 + a_8x_1x_3 + a_9x_2x_3 + a_{10}x_1x_2x_3 \quad (2)$$

where $y = R_s$ (or h) or $\log R_s$ (or $\log h$), and $x = M$ or $\log M$, where M is the molarity or normality of a buffer salt, ion-interaction reagent or proton.

According to the optimization range of the factor space, with an appropriate computation program the response surface, y , can be described as a function of the different variables and the optimum eluent compositions can be determined. Calculating the coefficients of eqn. 2, the plotting of the response surface and the selection of the optimum mobile phase conditions were performed on the CDC computer of the VUB/ULB computing center, using programs written in Fortran 77, as well as on a Macintosh (apple) computer, using programs written in Basic. Nevertheless, all these computations are valid only within the experimental limits considered. Extrapolation may lead to serious errors.

(3) The selection of the experimental points (x_1, x_2, x_3 corresponding to the values of the selected parameters; ion-interaction reagent concentration, buffer concentration and pH) in the factor space is also a critical part of the factor planning. Here too, preparative experiments will supply the required information. Mobile phase conditions which yielded satisfactory resolutions, peak heights and analysis times are the basic candidates to serve as an experimental point in the factor space. Other experimental points result from the variability range (+ or $-\Delta x_1, \Delta x_2$ and Δx_3) attributed to each parameter. Most often, new experiments have to be carried out under these mobile phase conditions. However, there are some practical limits to the range of variation attributed to each of the parameters, for example the solubility of tetrabutylammonium iodide or the pH limit of the Partisil ODS-3 stationary phase. In our case, the ion-interaction reagent concentration, buffer concentration and pH

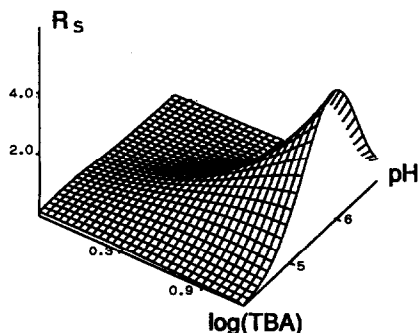


Fig. 7. Resolution surface for the separation of NO_2^- and Br^- peaks as a function of TBAI concentration (0.2–16 mM) and eluent pH (4–7). KHP concentration: 1 mM (see ref. 25).

ranged between 0.5 to 16 mM, 0.5 to 2 mM and 3.5 to 7.0, respectively. As a consequence the experimental point showing the best results cannot always be set at the centre of the central composite design.

Model fitting and prediction

In Table I, the fifteen mobile phase compositions according to the three-factor central composite design as well as the corresponding experimental resolutions and peak heights are shown. All results are the mean of three repeated experiments. To give an idea of the experimental uncertainty, ten replicate measurements at 8.0 mM TBAI, 1.0 mM KHP and pH 6.0 were carried out. The peak heights of Cl^- , NO_3^- and SO_4^{2-} showed relative errors of 4.7, 4.5 and 4.4%, respectively. The retention times of Cl^- , NO_3^- and SO_4^{2-} showed relative errors of 2.8, 1.8 and 1.7%, respectively. The experimental results in Table I were used for computer prediction of the resolution and peak height functions by polynomial fitting with eqn. 2. Predicted results were compared to the experimental values. The differences are relatively large, which is a confirmation of the too large confidence interval for the eleven polynomial coefficients of eqn. 2. In order to improve this situation, six additional experiments, selected on the basis of the results of the former optimization, were carried out. One of the advantages of a least squares regression method is that the more experimental data are available, the better the polynomial function will predict the experimental data and the smaller the confidence interval of the regression coefficients will be. The new computation confirmed that the results predicted with the 21 experimental data points are better than the results predicted with the 15 points. Differences between experimental and computed values are smaller than in the previous optimization

TABLE I

MOBILE PHASE COMPOSITIONS AND THE CORRESPONDING EXPERIMENTAL RESOLUTIONS AND PEAK HEIGHTS ACCORDING TO THE EXPERIMENTS OF THE THREE-FACTOR CENTRAL COMPOSITE DESIGN

TBAI (mM)	KHP (mM)	pH	Experimental		Experimental peak height (cm)		
			$R_s(\text{NO}_2^- - \text{Br}^-)$	$R_s(\text{NO}_3^- - \text{SO}_4^{2-})$	Cl^-	NO_3^-	SO_4^{2-}
8.0	2.0	6.0	1.11	0	11.50	3.00	6.50
8.0	2.0	4.0	0	1.12	14.00	5.50	7.20
8.0	1.0	6.0	1.35	1.52	9.90	9.20	16.20
8.0	1.0	4.0	0.73	3.34	11.30	6.80	9.0
4.0	2.0	6.0	1.00	0.97	10.00	4.50	10.20
4.0	2.0	4.0	0	2.51	13.50	5.50	5.20
4.0	1.0	6.0	1.33	3.57	8.50	4.00	7.50
4.0	1.0	4.0	0.81	7.23	14.00	5.50	5.40
8.5	1.5	5.0	0.89	1.11	13.80	5.80	8.70
3.5	1.5	5.0	0.67	5.00	9.00	3.30	2.90
6.0	2.1	5.0	0.69	1.53	8.10	2.90	4.50
6.0	0.9	5.0	0.94	4.00	12.80	6.00	10.50
6.0	1.5	6.2	1.06	0.77	11.90	5.10	9.70
6.0	1.5	4.0	0	3.76	8.20	3.80	4.00
6.0	1.5	5.0	0.64	2.67	7.30	3.30	4.10

calculation. In addition, an evaluation of different models corresponding to small changes in eqn. 2 (for example, with or without the last term $x_1x_2x_3$, of with or without the "log" form) were also carried out. As shown in Table II, the best result (smallest difference between predicted and experimental measured results) were obtained with a 21 data points prediction using the complete polynomial equation (no log form). An average deviation of 6.5% relative to the experimental results is observed. The fifteen data points prediction using the complete polynomial equation yields a larger average deviation of 14.1%. Predictions without the last term in eqn. 2 ($x_1x_2x_3$), or with the variables in the "log" form, resulted in more serious prediction errors. The results given in Table II indicate that, in the case of the three-factors optimization, with R_s or h as the response function y , the interaction term ($a_{10}x_1x_2x_3$) in eqn. 2 plays an important rôle and cannot be neglected. Similar results were also found in the prediction of the resolution $R_s(\text{NO}_3^- - \text{SO}_4^{2-})$ and the prediction of the peak heights of Cl^- , NO_3^- and SO_4^{2-} (results not shown). The 21 mobile phase composition values as well as the corresponding experimental and computer predicted resolutions and peak

TABLE II

COMPARISON BETWEEN THE EXPERIMENTALLY DETERMINED AND THE PREDICTED RESOLUTION $R_s(\text{NO}_2^- - \text{Br}^-)$ VALUES FROM 21 AND 15 EXPERIMENTAL POINTS WITH DIFFERENT MODELS

TBAI (mM)	KHP (mM)	pH	Experimental $R_s(\text{NO}_2^- - \text{Br}^-)$	Predicted $R_s(\text{NO}_2^- - \text{Br}^-)$			
				21 points simulation	15 points simulation	21 points without $x_1x_2x_3$	21 points no $x_1x_2x_3$ but with log term
8.0	2.0	6.0	1.11	1.18	1.15	5.30	4.70
8.0	2.0	4.0	0.00	0.00	0.06	2.72	0.00
8.0	1.0	6.0	1.35	1.32	1.32	3.39	1.60
8.0	1.0	4.0	0.73	0.66	0.67	2.04	0.11
4.0	2.0	6.0	1.00	0.99	1.02	3.06	0.33
4.0	2.0	4.0	0.00	0.00	0.03	1.40	0.00
4.0	1.0	6.0	1.33	0.81	1.28	1.84	0.22
4.0	1.0	4.0	0.81	0.74	0.71	1.43	0.32
8.5	1.5	5.0	0.89	0.80	0.78	3.54	0.48
3.5	1.5	5.0	0.67	0.59	0.74	1.75	0.18
6.0	2.1	5.0	0.69	0.64	0.52	3.22	0.13
6.0	0.9	5.0	0.94	0.98	1.08	2.28	1.50
6.0	1.5	6.2	1.06	1.02	0.95	3.45	0.95
6.0	1.5	4.0	0.00	0.30	0.11	1.85	0.00
6.0	1.5	5.0	0.64	0.74	0.59	2.86	0.35
6.0	0.5	6.0	1.00	1.29	1.74	2.06	0.48
4.0	1.0	5.0	1.03	0.86	1.06	1.72	1.12
2.0	1.0	4.2	0.74	0.72	1.12	1.10	0.75
1.0	1.0	5.65	0.00	0.40	1.80	0.65	0.03
1.0	2.0	4.05	0.00	0.00	0.72	0.32	0.00
0.5	1.0	5.0	0.54	0.60	1.80	0.64	0.05
Average relative deviation (%) ^a				6.50	14.06	> 30	31

^a for 17 data.

TABLE III
 TWENTY-ONE MOBILE PHASE COMPOSITIONS AND THE CORRESPONDING EXPERIMENTALLY DETERMINED AND COMPUTER PRE-
 DICTED RESOLUTIONS AND PEAK HEIGHTS

TBAI (mM)	KHP (mM)	pH	$R_s(\text{NO}_2^- - \text{Br}^-)$		$R_s(\text{NO}_3^- - \text{SO}_4^{2-})$		Peak height (cm)		Cl^-	NO_3^-		SO_4^{2-}	
			exptl.	pred.	exptl.	pred.	exptl.	pred.		exptl.	pred.	exptl.	pred.
8.0	2.0	6.0	1.11	1.18	0	0	11.50	11.97	11.50	3.00	3.59	6.50	6.61
8.0	2.0	4.0	0	0	1.12	1.68	14.00	12.70	14.00	5.50	4.91	7.20	6.68
8.0	1.0	6.0	1.35	1.32	1.52	2.21	9.90	10.90	9.90	9.20	7.83	16.20	13.40
8.0	1.0	4.0	0.73	0.66	3.34	3.80	11.30	11.80	11.30	6.80	6.50	9.0	8.94
4.0	2.0	6.0	1.00	0.99	0.97	1.29	10.00	9.41	10.00	4.50	4.13	10.20	9.09
4.0	2.0	4.0	0	0	2.51	2.04	13.50	11.35	13.50	5.50	4.50	5.20	3.45
4.0	1.0	6.0	1.33	0.81	3.57	3.31	8.50	7.75	8.50	4.00	3.83	7.50	11.33
4.0	1.0	4.0	0.81	0.74	7.23	6.61	14.00	12.39	14.00	5.50	6.55	5.40	5.94
8.5	1.5	5.0	0.89	0.80	1.11	1.81	13.80	10.84	13.80	5.80	5.93	8.70	7.21
3.5	1.5	5.0	0.67	0.59	5.00	3.59	9.00	8.82	9.00	3.30	4.73	2.90	5.39
6.0	2.1	5.0	0.69	0.64	1.53	1.44	8.10	10.33	8.10	2.90	3.40	4.50	5.68
6.0	0.9	5.0	0.94	0.98	4.00	4.22	12.80	9.70	12.80	6.00	5.33	10.50	9.13
6.0	1.5	6.2	1.06	1.02	0.77	0.80	11.90	10.48	11.90	5.10	4.40	9.70	9.20
6.0	1.5	4.0	0	0.30	3.76	3.16	8.20	12.16	8.20	3.80	5.14	4.00	5.34
6.0	1.5	5.0	0.64	0.74	2.67	2.55	7.30	9.99	7.30	3.30	4.46	4.10	6.42
6.0	0.5	6.0	1.00	1.29	5.11	4.61	7.40	8.78	7.40	3.40	6.00	16.10	17.65
4.0	1.0	5.0	1.03	0.86	4.98	5.29	8.80	8.94	8.80	8.20	4.89	12.50	7.79
2.0	1.0	4.2	0.74	0.72	7.86	7.51	10.20	11.06	10.20	8.20	8.23	4.50	4.21
1.0	1.0	5.65	0	0.40	5.52	5.85	4.30	5.26	4.30	4.30	4.94	11.50	8.50
1.0	2.0	4.05	0	0	2.22	2.84	7.80	9.77	7.80	6.20	7.09	3.00	5.08
0.5	1.0	5.0	0.54	0.60	7.20	7.64							
Average relative deviation (%)			6.5		7.0		10.7			11.5		13.2	
			(17 data)		(16 data)		(15 data)			(15 data)		(14 data)	

TABLE IV

OPTIMUM REGRESSION COEFFICIENTS OF EQN. 2 WITH 21 EXPERIMENTAL POINTS

	$R_{s1}(\text{NO}_2^- - \text{Br}^-)$	$R_{s2}(\text{NO}_3^- - \text{SO}_4^{2-})$	$h_1(\text{Cl}^-)$	$h_2(\text{NO}_3^-)$	$h_3(\text{SO}_4^{2-})$
a_0	3.543	32.070	69.589	48.218	30.690
a_1	-0.432	-3.812	-3.446	-6.307	-1.000
a_2	-4.060	-21.024	-13.590	-16.234	-24.550
a_3	-0.072	-1.837	-18.119	-10.022	-7.840
a_4	-0.005	0.025	-0.027	0.138	-0.019
a_5	0.283	1.141	0.047	-0.330	3.936
a_6	-0.081	-0.330	1.131	0.295	0.850
a_7	0.176	1.987	1.750	2.615	2.442
a_8	0.117	0.554	0.780	1.131	0.479
a_9	0.620	2.656	2.615	3.678	2.505
a_{10}	0.043	-0.346	-0.316	-0.625	-0.596

heights are summarized in Table III. The regression coefficients of eqn. 2 resulting from the optimization calculations with the 21 data points are presented in Table IV. The prediction error, *i.e.*, the difference, between the computed and experimental results is lower for the resolution (6.5–7.0%) than for the peak height (10.7–13.2%).

Table III indicates that the average prediction error for the resolutions $R_s(\text{NO}_2^- - \text{Br}^-)$ and $R_s(\text{NO}_3^- - \text{SO}_4^{2-})$ is about 6.5 and 7.0% (21 experimental data points). This is an acceptable magnitude. Fig. 8 shows the predicted and experimen-

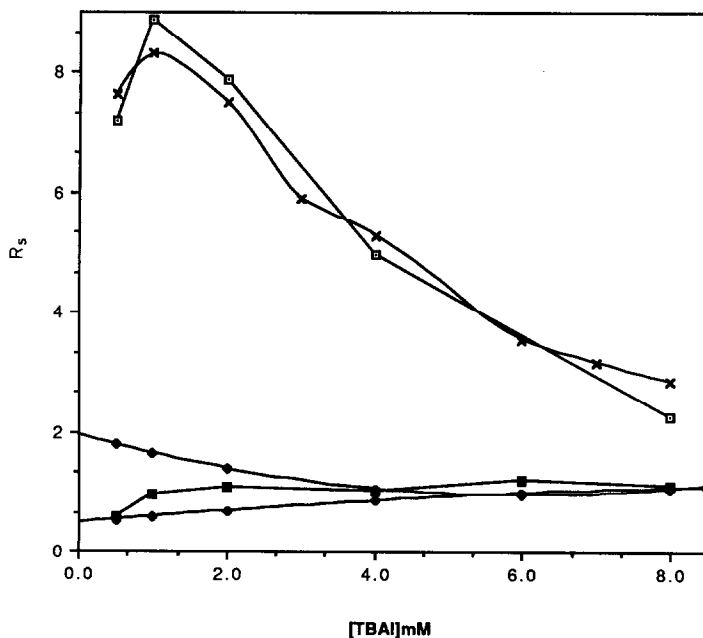


Fig. 8. Comparison between the experimental resolution factor curve and the predicted curves as a function of the TBAI concentration. KHP concentration: 1 mM (pH 5). (\square) exp. R_{s2} ; (\blacklozenge) pred. R_{s1} (21); (\times) pred. R_{s2} ; (\diamond) pred. R_{s1} (15); (\blacksquare) exp. R_{s1} .

tally measured resolution curves as a function of the TBAI concentration in the eluent. The predicted $R_{s1}(\text{NO}_2^- - \text{Br}^-)$ and $R_{s2}(\text{NO}_3^- - \text{SO}_4^{2-})$ data were computed with the 21 experimental data points. Predicted curves are close to the experimental curves especially at TBAI concentrations larger than 2 mM. A slightly improved prediction of $R_{s1}(\text{NO}_2^- - \text{Br}^-)$ is obtained with the 21 experimental data points than with the 15 (Fig. 8).

Deviations between predicted and experimentally measured curves with respect to the peak heights of Cl^- , NO_3^- and SO_4^{2-} , however, are slightly larger. Perhaps eqn. 2 is somewhat less suited to approximate the peak heights function than it does the resolution functions.

The mobile phase optimization

Table V gives the computed resolution values of the critical peak pair $\text{NO}_2^- - \text{Br}^-$ (R_{s1}) which are higher than the resolution criterion of 1.35 as a function of the mobile phase conditions. With a step size of 0.5 mM, the computation was started at a TBAI concentration of 0.5 mM and stopped at 8.5 mM, but extends over the whole factor domain of buffer concentration (0.5 to 2 mM with a step size of 0.5 mM) and pH (4 to 6.5 with a step size of 0.5 pH units). The results of these predictions indicate that to satisfy the preset condition ($R_{s1} > 1.35$) for the resolution of $\text{NO}_2^- - \text{Br}^-$ the mobile phase TBAI concentration must be higher than 6 mM, the KHP concentration must, on the whole, not exceed 1 mM, and the pH must preferably be in the range of 5 to 6.5. Only a few exceptions have been observed: four points with a KHP concentration larger than 1.0 mM (1.5–2) but accompanied by higher pH values (6.5), and five other points with a pH value lower than 5 (4.5) and a KHP concentration below 1 mM (0.5 mM). Computed mobile phase compositions yielding $\text{NO}_3^- - \text{SO}_4^{2-}$ resolution values (R_{s2}) between 1.5 and 1.8 are also given in Table VI. Only six mobile phase compositions satisfy this requirement. On the whole, there is only one condition which meets both the adequate resolution for peak pair $\text{NO}_2^- - \text{Br}^-$ ($R_{s1} > 1.35$) and the constrained resolution value for $\text{NO}_3^- - \text{SO}_4^{2-}$ ($1.5 < R_{s2} = 1.62 < 1.8$). The corresponding values of the three variables are $[\text{TBAI}] = 8.5 \text{ mM}$, $[\text{KHP}] = 1 \text{ mM}$ and pH 6.

As a general rule, higher TBAI concentration, higher pH and lower KHP concentration are required to obtain high peak heights for nitrate and sulphate. Optimum chloride peak heights correspond in the optimization factor domain to all TBAI and KHP concentrations but at a somewhat lower pH. The global optimum conditions, therefore, were obtained at a mobile phase composition of $[\text{TBAI}] = 8$ to 8.5 mM, $[\text{KHP}] = 0.5$ to 1.0 mM and pH 6 to 6.5. According to these values, the critical resolution value of peak pair NO_2^- and Br^- (being larger than 1.35) was guaranteed and thus a baseline separation of this peak pair was attained. At the same time an high detection response for peaks NO_3^- and SO_4^{2-} and an overall elution time within 10 min (the resolution of NO_3^- and SO_4^{2-} is in the range of 1.5 to 1.8) can also be obtained. This result coincides very well with the best result we observed experimentally (R_s of $\text{NO}_2^- - \text{Br}^- = 1.33$ to 1.38, analysis time = 9 min and high detection response for NO_3^- , SO_4^{2-} and Cl^-) for a mobile phase composition of $[\text{TBAI}] = 8 \text{ mM}$, $[\text{KHP}] = 1 \text{ mM}$ and pH 6.

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