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Computer-assisted predictions of resolution, peak height and retention time for the separation of inorganic anions by ion chromatography

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ABSTRACT

A computer-assisted method, designed to determine the combined effects of mobile phase variables, was applied in an experimental study in which both an inorganic anion-exchange chromatographic system and a reversed-phase ion-interaction chromatographic system were tested. A Hamilton PRP-X100 anion-exchange column and a Partisil 10 ODS-3 reversed-phase column were used in these studies. Direct resolution, sensitivity (peak height) and retention time modelling were used to predict the total analysis time and the resolutions and peak heights of some critical peaks in the search area of the mobile phase composition. The relationship between resolution, retention time and peak height and the mobile phase variables was approximated by a quadratic polynomial function. In this work, the results of computer plotting of some three-dimensional response surfaces are presented. These graphical illustrations not only helped us to provide a detailed understanding of the chromatographic processes, but also enabled us to define more precisely the location of the optimum conditions.

INTRODUCTION

Computer optimization procedures, using computer programs to select chromatographic conditions leading to the achievement of a desired separation, have found extensive application in liquid chromatography [1,2]. One of the most successful of these applications is the selection of mobile phase composition for reversed-phase liquid chromatography (RPLC). Despite the success experienced with RPLC the knowledge gained has found only limited application to ion chromatography (IC) for the separation of inorganic anions within both ion exchange [3,4] and reversed phase ion-pair or ion-interaction chromatography [5]. Likely reasons are as follows: (1) The retention mechanism involved in ion-exchange and ion-interaction chromatography is more complex than the binary or ternary solvent mixture system in HPLC. As a consequence, an empirical, mathematical relationship between solute retention and mobile phase composition may be required. (2) Lack of facilities to control optimization with respect to the real analytical importance of individual peaks and/or the time of analysis. The aim of optimization of eluent composition in some particular cases cannot simply be defined as the maximization of the resolution (R_s) of all adjacent solute peaks and consideration of a reasonable analysis

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time. Under this circumstance, the choice of a general quality criterion is fraught with problems, since the requirements are often ambiguous and difficult to express in quantitative terms. (3) Furthermore, attempting to optimize many criteria when dealing with a complex sample often complicates the process. An example was given in a previous optimization problem, where the objective was to achieve a global optimum [5]. This required the maximization of the separation of the pair of peaks NO_2^- and Br^-, which is currently the most difficult to resolve, but simultaneously this optimization also required minimization of the resolution of peaks NO_3^- and SO_4^{2-} in order to reduce the total analysis time, and to increase the detection sensitivity (peak height) as much as possible. Three criteria were thus used in this optimization exercise. Such a typical optimization example occurs in practical chromatographic separation problems and, thus, a method such as the widely used "mixture design statistical technique" (MDST) [6], or a method involving composite criteria such as the overlapping resolution map (ORM) concept [7] or the chromatographic optimization function (COF) [8,9] describing the quality of the chromatogram as a whole, might be difficult to apply here.

Most computer-assisted optimization procedures are based on retention modelling [4,10,11]. The retention model that describes the relationship between retention time (t_R) (or capacity factor) and mobile phase composition allows retention times to be predicted for any mobile phase in the search area. The reliability of these predicted retention times is dependent on the suitability of the retention model used. Mechanistic, semiempirical or fully empirical retention models are frequently used to tackle a specific separation problem [12]. Owing to the complex nature of inter- and intramolecular interactions encountered in retention phenomena, a multidimensional empirical modelling technique based on polynomial regression will be the method of choice. This technique is very adaptable to different retention mechanisms and enables several variables to be handled simultaneously. In addition, it can deal with irregularly spaced variable data. In this paper, we present the computer-assisted results of some experimental studies dealing with both inorganic anion-exchange chromatography and reversed-phase ion-interaction chromatography designed to determine the combined effects of mobile phase variables. A Hamilton PRP-X100 anion-exchange column and a Partisil 10 ODS-3 reversed-phase column were used in these studies. Direct resolution, detectability (peak height) and retention time modelling were used to predict the total analysis time and the resolutions and the peak heights of some critical peaks in the search area of the mobile phase composition. The relationship between resolution, retention time and peak height and the mobile phase variables was approximated by a quadratic polynomial function. The computer plotting of some three-dimensional response surfaces not only helped to provide a detailed understanding of the chromatographic process but also led to a more precise definition of the location of the optimum conditions.

EXPERIMENTAL

Apparatus

A DuPont 870 HPLC pump, a Valco injection valve equipped with a 100-ml loop, a Perkin-Elmer LC-21 conductivity detector and an Ommiscribe B5 217-5 strip chart recorder were used. One 250 × 3.9 mm I.D. PRP-X100 anion-exchange column (Hamilton) with a cartridge guard column and one 250 × 4.6 mm I.D. Partisil 10 ODS-3 RP column (Whatman) with a 60 × 2.1 mm I.D. guard column were used in the experiments.

Reagents

Methanol, chromatographic quality, was obtained from Merck (Germany). Hydroxybenzoic acid (HBA, analytical-reagent grade) was from Aldrich-Chemie (Germany). Tetrabutylammonium iodide (TBAI), potassium hydrogen phthalate (KHP) and other reagents are similar to the description in ref. 5.

All separations were carried out at room temperature, and the separation column, guard column and detector cell were thermally isolated in a wooden box to minimize short-term temperature variations. Each retention time (t_R) and peak height (h) is the mean from two experiments.

Statistical optimization methods

The mobile phase optimization we applied can be considered as a stochastic statistical procedure. The essential steps of this approach, the choice of the optimization criteria and the choice of the factors have been discussed in a previous publication [5].

In this work an anion-exchange chromatographic mode and a reversed-phase ion-interaction mode were chosen to investigate the effect of mobile phase variables on the chromatographic response (resolution, peak height and/or retention time). Both regular and irregular experimental data were fitted by a statistical model.

Anion-exchange chromatography

The original separation method, recommended by the PRP-X100 column manufacturer, allows the separation of F^-, CO_3^{2-}, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-} and SO_4^{2-} using an eluent of 4 m*M* HBA at pH 8.6; it needs about 16 min to complete the total elution with a mobile phase flow-rate of 3 ml/min. The aim of the optimization is to reduce the total elution time with the objective of baseline separation of all the anions mentioned before. By slightly increasing or decreasing the hydroxybenzoic acid (HBA) concentration and the pH value based on the mobile phase composition prescribed by the manufacturer, the aim of optimization might be achieved since we found that the retention of all these anions, and in particular of the last three relatively strongly retained species, NO_3^-, HPO_4^{2-} and SO_4^{2-}, is very sensitive to changes in the HBA concentration and pH. In addition, the peaks of HPO_4^{2-} and NO_3^-, which are the first two peaks, tend to overlap and thus form a critical part of the optimization.

A small amount of organic modifier (methanol) was added to the mobile phase to optimize the retention selectivity of the above-mentioned anions, because we found that the organic modifiers are also very sensitive to the retention behaviour of all these anions. It is known that PRP-X100 is a low-capacity, strong-base anion-exchange phase derived from a macroporous poly(styrene–divinylbenzene) reversed phase, PRP-1. It has retained some of the original reversed-phase characteristics of its parent to function in that mode [16].

A two-factor, three-level factorial design (3^2) was adopted as the optimization strategy. A set of nine experimental points was used to investigate the effect of the pH and methanol content of the mobile phase on the resolution and the total elution time. A second set of eleven experimental points was used to investigate the effect of the HBA concentration and pH on the resolution and the total elution time. These results were used for the construction of the three-dimentional plots of resolution and retention time surfaces. The compositions of the mobile phase that were used in this work are given in Table I.

Ion-interaction chromatography

ODS-3 packing is an octadecyl-bonded reversed phase. It can be applied to separate common inorganic anions when the packing is coated or dynamically coated with a hydrophobic quaternary ammonium salt. The quaternary ammonium salt is commonly presented together with a competing anion (such as phthalate) in the mobile phase. In this case, the ion-interaction reversed-phase chromatographic system has some ion-exchange characteristics. However, the ion-exchange capacity is very low and organic modifiers must not be used to optimize the retention selectivity of inorganic anions. The concentrations of TBAI and KHP and the pH of the mobile phase were selected as the factors that will be used to optimize the selected criteria [14].

Two sets of thirteen and nine experimental points were selected from a series of evolutionary experiments. The first set of thirteen experimental points covered two factors, each at six levels (TBAI concentration, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 m*M*; and pH, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5). The second set of nine experimental points covered two factors at four and five levels (TBAI concentration 2, 4, 6 and 8 m*M*; and KHP concentration 0, 0.5, 1.0, 1.5 and 2.0 m*M*). These experimental points represented almost the whole factor space in which we were interested, but by a limited number of experimental points. Furthermore, most of these selected mobile phase compositions resulted in a reasonable separation of the two critical peak pairs (NO_2^-–Br^- and NO_3^-–SO_4^{2-}), as well as in a measurable peak height. These results were used for the construction of the three-dimensional plots of the resolution and peak-height surface.

For the ion-interaction chromatography, we used two sets of experimental data which existed from "one factor at a time" experiments without any previous factorial design. In addition, (1) a two-factor,

TABLE I

THE RESULTS OF THE COMBINED EFFECT OF TWO FACTORS IN ANION-EXCHANGE CHROMATOGRAPHY ON THE RESOLUTION AND THE RETENTION TIME CALCULATION OF THE OPTIMUM REGRESSION COEFFICIENTS USING EQN. 2

	HBA[a] (m*M*)	pH[a]	R_s (HPO_4^{2-}–NO_3^-)	t_R(SO_4^{2-})	Methanol[b]	pH[b]	R_s (HPO_4^{2-}–NO_3^-)	t_R(SO_4^{2-})
1	3.0	8.2	11.04	24.0	1.8	7.5	10.95	23.3
2	3.0	8.8	8.07	18.6	2.4	7.5	11.60	22.0
3	3.0	9.4	5.44	14.7	3.0	7.5	10.36	21.9
4	4.0	8.2	9.95	19.5	1.8	8.5	5.46	13.6
5	4.0	8.8	5.71	13.4	2.4	8.5	4.86	12.4
6	4.0	9.4	2.35	9.5	3.0	8.5	3.29	10.8
7	5.0	8.2	5.91	13.5	1.8	9.5	0.56	7.8
8	5.0	8.8	3.60	10.0	2.4	9.5	1.05	8.3
9	5.0	9.4	1.87	8.1	3.0	9.5	1.28	7.6
10	4.0	9.8	1.28	7.2				
11	4.0	7.5	10.36	22.0				
a_0			−12.28	0.25			−3.39	6.39
a_1			6.30	−0.72			−3.53	0.51
a_2			3.07	0.55			1.65	−1.03
a_3			−0.66	0.16			−5.03	−1.50
a_4			−0.17	−0.04			−0.15	0.05
a_5			−0.81	−0.07			0.87	0.04

[a] The mobile phase has a constant methanol content of 3%.

[b] The mobile phase has a constant HBA concentration of 4 m*M*.

six-level or five-level design would lead to a much too large number of experiments (36 or 25) to be carried out and (2) also, among these experiments, too many useless results (bad separation results) could be expected. The compositions of the mobile phase that were used in this work are given in Table II.

The response function

The mathematical form of the response functions, *f*, relating the observed response, *y* (the resolution factors, peak heights and retention time), to the chromatographic variables, x_n, is unknown:

$$y = f(x_n) \tag{1}$$

In order to describe the response surface in the region where the optimum is to be found by means of a graphic plotting method, the response surface was approximated by a generalized polynomial equation of the second order. When the responses are expressed as a function of two independent variables, the polynomial equations can be described by a quadratic equation:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1^2 + a_4x_2^2 + a_5x_1x_2 \tag{2}$$

In the case of the ion-exchange chromatography, $y = \log R_s$ (or $\log t_R$), $x_1 = \log [M]$, M = HBA, x_2 = pH, when the fitting was carried out on eleven experimental data points and $y = \log R_s$ (or t_R), x_1 = log(MeOH%), x_2 = pH, when the fitting was performed on the nine experimental data points. In the case of the ion-interaction chromatography, $y = \log R_s$ (or $\log h$), $x_1 = \log [M]$, M = TBAI, x_2 = pH, when the fitting was carried out on thirteen experimental data points and $y = R_s$ (or h), x_1 = (TBAI), x_2 = (KHP), when the fitting was performed on the nine experimental data points.

The optimization and estimation of the coefficients of the polynomial equation

The optimization procedure deals with the esti-

TABLE II

THE RESULTS OF THE COMBINED EFFECT OF TWO FACTORS IN REVERSED-PHASE ION-INTERACTION CHROMATOGRAPHY ON THE RESOLUTION AND THE PEAK-HEIGHT CALCULATION OF THE OPTIMUM REGRESSION COEFFICIENTS USING EQN. 2

	[TBAI] (m*M*)	pH	[KHP] (m*M*)	$R_{s1}(NO_2^-–Br^-)$	$R_{s2}(NO_3^-–SO_4^{2-})$	$h_1(Cl^-)$ (cm)	$h_2(NO_3^-)$ (cm)	$h_3(SO_4^{2-})$ (cm)
1	0.50	5.00	1	0.60	7.20	7.8	6.2	3.0
2	1.00	5.50		0	5.52	4.3	4.3	11.5
3	1.00	4.00		0	3.1	3.3	3.2	5.2
4	2.00	4.00		0.74	7.87	10.2	8.2	4.5
5	2.00	5.50		1.10	6.41	9.3	7.1	5.9
6	4.00	4.00		0.81	7.23	14.0	5.5	5.4
7	4.00	5.00		1.03	4.98	8.8	8.2	12.5
8	4.00	6.00		1.33	3.57	8.5	4.0	7.5
9	8.00	4.00		0.73	3.34	11.3	6.8	9.0
10	8.00	4.50		1.06	3.19	11.6	11.3	15.2
11	8.00	6.00		1.38	1.52	10.2	11.7	19.4
12	8.00	6.50		1.35	1.25	6.3.	8.2	15.8
13	16.00	5.00		1.11	2.10	10.1	14.9	13.2
a_0				−3.75	−1.52	−0.17	−2.30	−1.33
a_1				0.98	1.45	1.25	−0.62	0.24
a_2				1.18	0.87	0.33	1.30	0.75
a_3				0.38	−0.46	−0.14	0.18	−0.08
a_4				−0.11	−0.08	−0.03	−0.14	−0.07
a_5				0.02	−0.28	−0.18	0.16	0.05
1	2.00	6	1.00	1.05	4.79	8.1	5.8	5.6
2	4.00		0	1.25	–	6.5	3.5	–
3	4.00		1.00	1.33	3.57	8.5	4.0	7.5
4	4.00		2.00	1.00	0.97	10.0	3.5	6.0
5	6.00		0.50	1.00	5.11	7.4	3.4	16.1
6	6.00		1.50	1.06	0.77	11.9	5.1	9.7
7	8.00		1.00	1.38	1.52	10.2	11.7	19.4
8	8.00		1.00	1.13	1.60	9.9	5.2	11.0
9	8.00		2.00	1.10	0	11.5	3.3	6.5
a_0				1.25	2.25	4.11	4.99	0.92
a_1				−0.01	0.30	0.72	−1.82	3.23
a_2				−0.55	5.31	3.40	6.53	−0.52
a_3				0	0.02	−0.07	0.30	0
a_4				−0.10	−2.27	−1.28	−1.36	1.46
a_5				0.03	−0.21	−0.31	−0.84	−1.57

mation of the coefficients in the polynomial equation in order to describe as well as possible the responses of a chromatographic experiment. By using the information contained in the optimized equation the analyst can alter the dependent variables in the desired fashion and calculate wherever he wants, respecting the boundaries of the response value.

The polynomial eqn. 2 is an example of an expression describing the chromatographic dependence of the dependent variables (resolution factor, peak height , retention time, etc.) on the independent variables (concentration of the competing ions, percentage of organic modifiers, pH of the mobile phase, etc.). However, the relation between the response y and variables x_1 and x_2 in this model, obviously, is not a causal relation since it is based not on a theoretical (thermodynamic or mechanic) derivation but on the assumption that the resolution factor of two adjacent peaks or the peak height or retention time of any analyte within a LC system depends not only on each individual mobile phase variable but also on the interactions between these variables. It is clear that the validity and the quality of this model are highly dependent on how well the real but unknown coefficients of the equation can be estimated. In other words, the coefficients of the polynomial equation must be estimated using statistical methods before the optimal mobile phase composition can be calculated in the global optimization process. Since the accuracy of the chromatographic response prediction using this model is highly dependent on the quality of the estimation of the coefficients in eqn. 2, it is thus highly dependent on the algorithms and the computing program chosen. The coefficients in eqn. 2 have been estimated and optimized employing the least-squares approach with a relatively small set of experiments and using a computer program that involves a most important subroutine called VAO5A.

The subroutine VAO5A

Since the key to the global optimization process of the mobile phase composition is to optimize the coefficients of eqn. 2, the subroutine VAO5A was employed in order to obtain the optimum coefficients. The original VAO5A program was obtained from the Harwell Subroutine Library (Hopper, 1978) [15], and it is now running on the CDC-6500 computer of the Vrije Universiteit Brussel — Université Libre de Bruxelles (V.U.B.–U.L.B.) computer centre. It can be used in cases where minimization of a sum of squares is required. The program is a compromise between three different algorithms for minimizing a sum of squares, namely the Newton–Raphson, the steepest descent and the Marquadt algorithms. It automatically obtains and improves an approximation of the Jacobian J (the first-derivative matrix), following the ideas of Broyden. The VAO5A algorithm is unable to deal with constraints, but a reasonable choice of the parameter DSTEP and of the initial values of the parameters prevents the program from leaving the feasible parameter space region.

The standard output of VAO5A provides only the values of the residuals (functions) and the parameters (a_0, a_1, a_2, a_3, a_4 and a_5 in eqn. 2). In order to provide valuable elements for the statistical evaluation of the coefficients' optimization, the VAO5A program was supplemented with a program called ERROR 11, allowing the computation of the correlation matrix and confidence regions. The Jacobian and Hessian matrices, as well as the 95% confidence limits of the parameters, may also be obtained. Detailed discussions concerning these algorithms and the computer programs can be found in ref. 16.

After the coefficients in eqn. 2 have been estimated, a three-dimensional response surface can be plotted using "Mathematical" software on a Macintosh II computer. The optimum mobile phase composition can then, be either calculated with a small program using BASIC language or visualized graphically using the Macintosh computer.

RESULTS AND DISCUSSION

Based on the experimental results and the discussion reported in a previous publication [5], the simultaneous effects of two factors in the mobile phase on the resolutions of some critical peak pairs, on the retention time of the last peak and on the peak heights were investigated. Table I shows the compositions of the mobile phase with anion-exchange chromatography that have been used in the two-factor, three-level experimental design. Eleven (9 + 2) and nine experimental points were evaluated chromatographically. The duplicated experimental results of the measured retention time of the last peak SO_4^{2-} and the calculated resolution factors, R_s, for the critical peak pair of HPO_4^{2-} and NO_3^- were also reported in Table I for the eleven- and nine-point experiments, respectively. The variables in the eleven point experiments were the HBA concentration and the pH of the mobile phase, but the methanol content was kept constant. The two variables in the case of the nine-point experiments were the percentage of methanol and the pH value, but the HBA concentration in the mobile phase was constant. In order to predict the resolution factors and the retention times of some particular peaks, the visualized three-dimensional response surfaces and the ultimate global chromatographic optimal mobile phase composition, polynomial regressions were performed on the data from Table I according to eqn. 2. The best model was selected: a logarithmic form was used in the case of both [HBA]–pH

TABLE III

COMPARISON BETWEEN THE EXPERIMENTALLY DETERMINED AND THE PREDICTED RESOLUTION, R_s, (NO_3^-–HPO_4^{2-}) AND RETENTION TIME t_R (SO_4^{2-}) VALUES IN ELEVEN- AND NINE-POINT EXPERIMENTAL, RESPECTIVELY

	Resolution prediction (HPO_4^{2-}–NO_3^-)						Retention prediction (SO_4^{2-} peak)					
	[HBA]–pH			MeOH%–pH			[HBA]–pH			MeOH%–pH		
	Exptl.	Pred.	Deviation	Exptl.	Pred.	Deviation	Exptl.	Pred.	Deviation	Exptl.	Pred.	Deviation
1	11.04	11.75	0.71	10.95	12.59	1.64	24.00	24.65	0.65	23.30	23.99	0.58
2	8.07	8.63	0.56	11.60	11.85	0.25	18.60	19.15	0.55	22.00	22.86	0.86
3	5.44	4.78	−0.66	10.36	8.82	−1.54	14.70	13.90	−0.80	21.85	20.52	−1.33
4	9.95	8.61	−1.34	5.46	4.04	−1.42	19.50	17.75	−1.75	13.60	12.59	−1.01
5	5.70	5.50	−0.20	4.86	4.89	0.03	13.40	13.62	0.22	12.35	12.19	−0.16
6	2.35	2.65	0.30	3.29	4.42	1.13	9.50	9.77	0.27	10.08	11.03	0.75
7	5.91	6.55	0.64	0.56	0.66	0.10	13.50	13.87	0.37	7.80	8.22	0.42
8	3.60	3.75	0.15	1.05	1.02	−0.03	10.00	10.54	0.54	8.25	8.05	−0.20
9	1.87	1.62	−0.25	1.28	1.12	−0.16	8.10	7.48	−0.62	7.55	7.35	−0.20
10	1.28	1.39	0.11				7.20	7.54	0.34			
11	10.36	10.16	−0.20				22.10	22.20	0.10			

and % MeOH–pH regression. Table I shows the best non-linear least-squares estimated coefficients of the corresponding polynomial eqn. 2.

Table II contains duplicated results of measured peak heights of Cl^- (h_1), NO_3^- (h_2) and SO_4^{2-} (h_3), and calculated resolution factors (R_s) for two critical peak pairs (NO_2^-–Br^- and NO_3^-–SO_4^{2-}) corresponding to each of the irregular designed experi-

TABLE IV

COMPARISON OF THE PREDICTION QUALITY BETWEEN THE DIFFERENT EXPERIMENTAL STRATEGIES USING THE POLYNOMIAL REGRESSION MODELS

Experimental strategy	Prediction quality (R.S.D., %)						
	Resolution			Peak Height			Retention time Peak of SO_4^{2-} (last)
	R_{s1}[a]	R_{s2}[b]	R_{s3}[c]	Cl^-	NO_3^-	SO_4^{2-}	
Two-factor, three level design (9 + 2 exptl. data)			7.5 (out of 9 data)				4.1 (out of 11 data)
Two-factor, three level design (9 exptl. data)			9.18 (out of 7 data)				4.6 (out of 9 data)
Three-factor central composite design (15 + 6 exptl. data[d])	6.5 (17 data)	7.0 (16 data)		10.7 (15 data)	11.5 (15 data)	13.2 (14 data)	
Irregular exptl. data (13 points)	18.2 (9 data)	14.8 (9 data)		12.0 (9 data)	11.4 (10 data)	18.8 (9 data)	
Irregular exptl. data (9 poins)	9.2 (8 data)	>25 (7 data)		6.4 (8 data)	18.0 (7 data)	12.1 (8 data)	

[a] NO_2^-–Br^-.
[b] NO_3^-–SO_4^{2-}.
[c] HPO_4^{2-}–NO_3^-.
[d] R.S.D. (%) data taken from ref. 5.

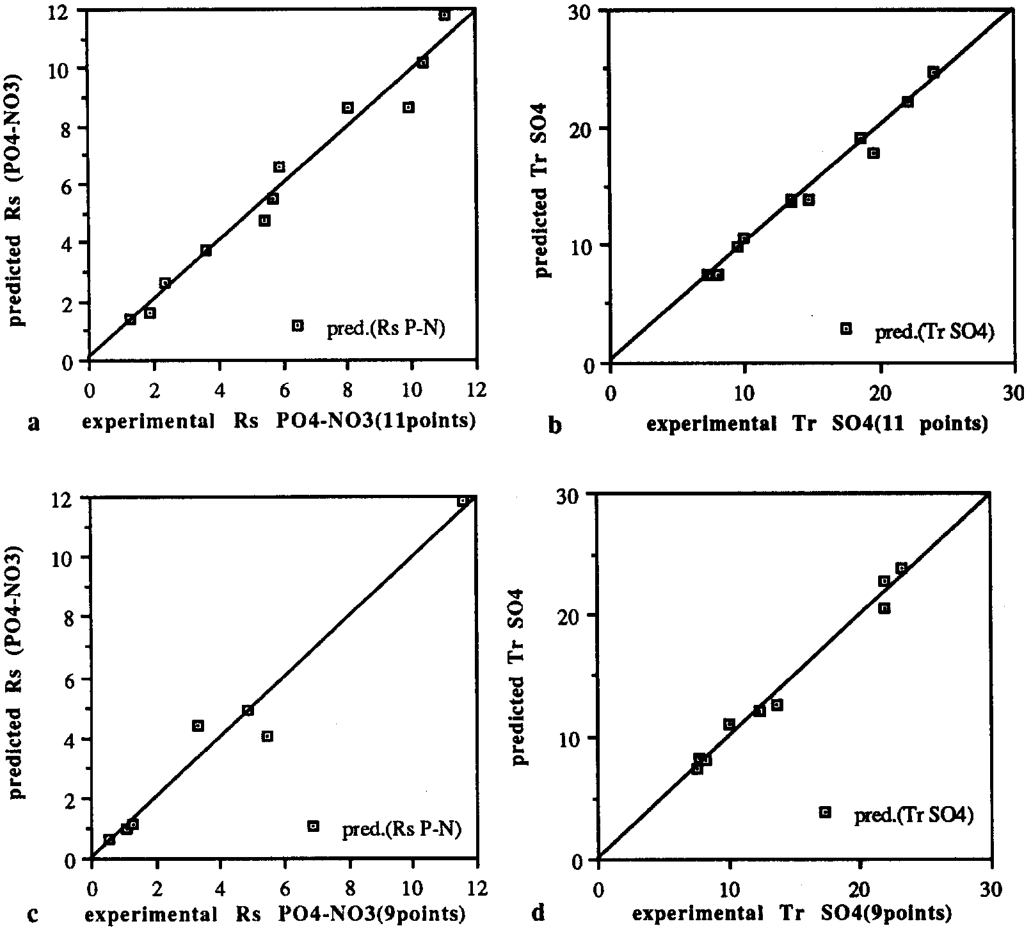


Fig. 1. The actual resolution factors of HPO_4^{2-}–NO_3^- and retention time of SO_4^{2-} obtained from the eleven- and nine-points experiments using the two-factor, three-level design plotted against the predicted resolution factors (a and c) and retention times (b and d). Lines: (a) $y = 8.07 \cdot 10^{-2} + 0.99x$, $R^2 = 0.97$; (b) $y = 0.10 + 0.99x$, $R^2 = 0.98$; (c) $y = 2.03 \cdot 10^{-2} + 0.99x$, $R^2 = 0.96$; (d) $y = 4.66 \cdot 10^{-2} + 1.00x$, $R^2 = 0.99$.

ments for the thirteen combinations of the variables [TBAI] and pH, and for the nine combinations of the variables [TBAI] and [KHP]. In these cases, a reversed-phase ion-interaction chromatographic system was used. Table II also shows the optimal non-linear least-squares estimated coefficients a_0, a_1, ... a_5. A logarithmic form of eqn. 2 in the case of variables [TBAI] and pH (thirteen experimental points) and the normal form of eqn. 2 in the case of variables [TBAI] and [KHP] (nine experimental points) was applied for the polynomial regressions.

Table III shows the resolution (R_s) and retention time (t_R) computer predictions based on the two-factor, three-level experimental design in an ion-exchange chromatographic system. In this table, the computer-predicted resolution R_s (HPO_4^{2-}–NO_3^-) and retention time t_R (SO_4^{2-}) values are compared with the experimentally determined values. In all cases, the deviations between experimental and predicted value are rather small. They vary between 1.9 and 13.5% for the HBA–pH effect on the resolution prediction, between 0.6 and 26% for the % MeOH–pH effect on the resolution prediction, between 0.5 and 9.0% for the HBA–pH effect on the retention time prediction and between 1.3 and 7.4% for the % MeOH–pH effect on the retention time predic-

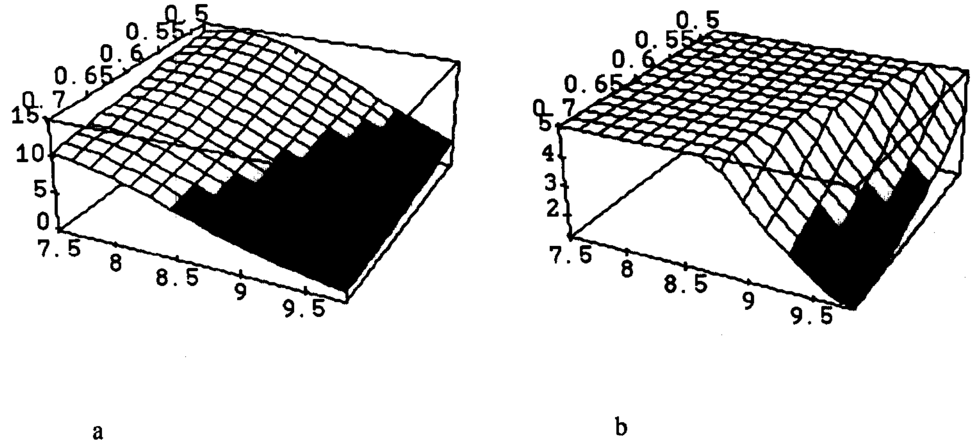


Fig. 2. (a) The resolution factor of the peak pair HPO_4^{2-}–NO_3^- as a function of HBA concentration and pH of the mobile phase with constant methanol content (3%). Separation column: anion-exchange Hamilton PRP-X100. Regression and plotting model: logarithmic form of eqn. 2. (b) Three-dimensional plot of resolution factor surface, R_s (HPO_4^{2-}–NO_3^-) with R_s ranging from 1.2 to 5.0. Other conditions are the same as in (a).

tion. This achievement of computer prediction is also shown in Table IV. Table IV compares the prediction qualities of the results when different experimental strategies are used. In all cases of the two-factor three-level experimental design (nine plus two experimental points and nine experimental points), the average relative standard deviation (R.S.D.) between the computer-predicted and the experimental values is rather low, and the retention time prediction is the best (R.S.D. < 5%).

Fig. 1a–1d shows the plots of the predicted resolution factors and retention times against the actual resolution factors of HPO_4^{2-}–NO_3^- and retention times of SO_4^{2-} based on the factor-designed eleven- and nine-point chromatographic evaluations. Linear correlations were obtained. Slopes of the regression lines are in the range 0.99–1.0. Their intercepts are in the range 0.02–0.1 and the correlation coeffi-

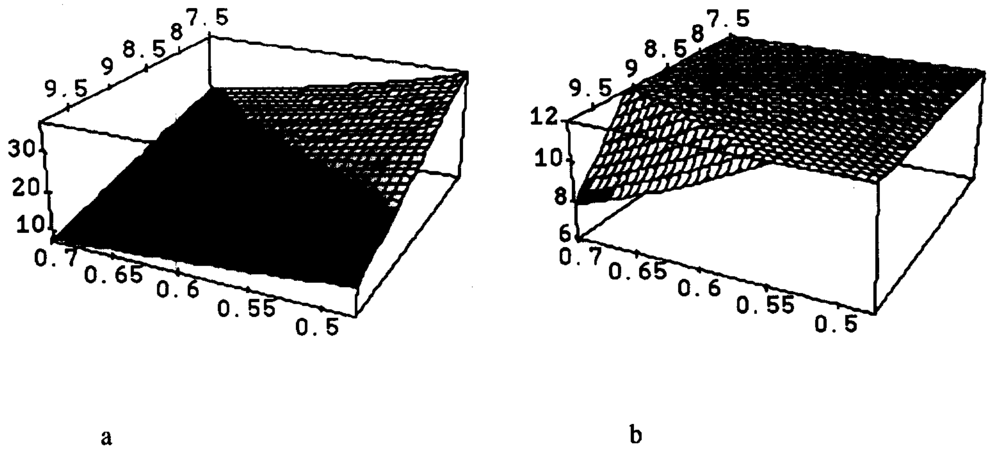
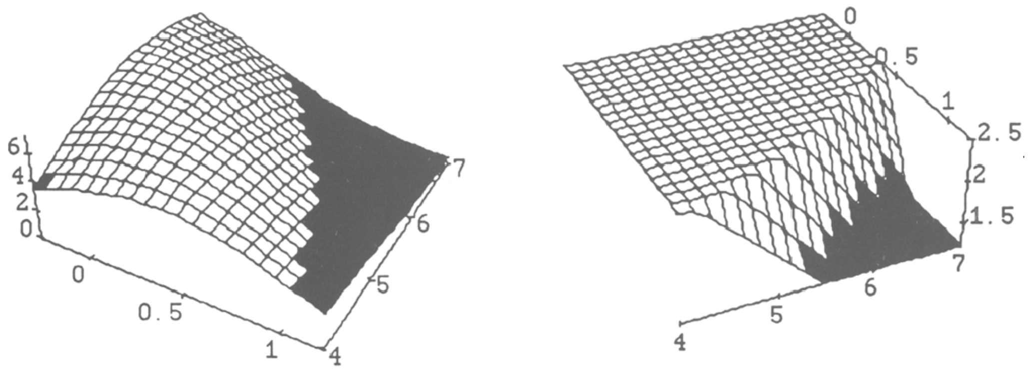


Fig. 3. (a) The retention time of the last peak, SO_4^{2-} as a function of HBA concentration and pH of the mobile phase with constant methanol content (3%). Separation column: anion-exchange Hamilton PRP-X100. Regression and plotting model: logarithmic form of eqn. 2. (b) Three-dimensional plot of retention time (t_R) of SO_4^{2-} with t_R ranging from 6 to 12 (min). Other conditions are the same as in (a).

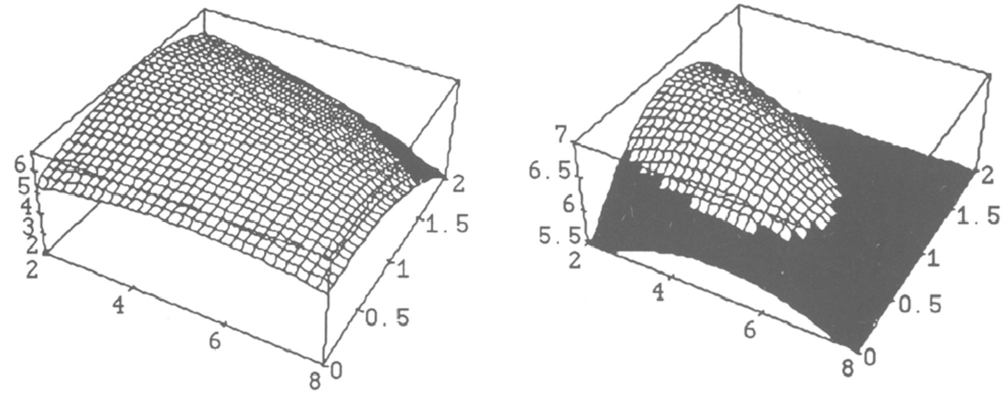


a b

Fig. 4. (a) The resolution surface of the peak pair R_s(NO_3^-–SO_4^{2-}) as a function of TBAI concentration and pH of the mobile phase with constant KHP concentration (1 m*M*). Separation column: Whatman Partisil 10 ODS-3 RP. Regression and plotting model: logarithmic form of eqn. 2. (b) Three-dimensional plot of resolution surface R_s (NO_3^-–SO_4^{2-}) with R_s ranging from 1.2 to 2.5. Other conditions are the same as in (a).

cients are very close to unity.

When an irregular experimental design was used, quite good agreement was obtained between the experimental and the predicted results: for example R_s NO_3^-–SO_4^{2-} and peak heights Cl^- and NO_3^- with TBAI and pH as variables (thirteen experimental points), and R_s NO_2^-–Br^- and peak heights Cl^- and SO_4^{2-} with TBAI and KHP as variables (nine experimental points). However, in the remaining cases using irregular experimental design, the differences between experimental results and predicted data were much larger. The reasons for the uncertainty of the predictions are (1) the experimental sets we used were not organized in a proper factorial design and (2) the number of experimental points used was too small to obtain a good least-squares estimation of the coefficients corresponding to eqn. 2. There are several advantages of organizing the experiments in a factorial design [17]: (i) When there are no interactions the factorial design



a b

Fig. 5. (a) The peak height of Cl^- as a function of TBAI and KHP concentrations in the mobile phase with constant pH (5). Separation column: Whatman Partisil 10 ODS-3 RP. Regression and plotting model: normal form of eqn. 2. (b) Three-dimensional plot of peak height surface h (Cl^-) with h range from 5.5 to 7.0 (cm). Other conditions are the same as in (a).

gives the maximum efficiency in the estimation of the effects. (ii) When interactions exist, and their nature is unknown, a factorial design is necessary to avoid misleading conclusions. (iii) In the 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.

Figs. 2a–5a show some typical computer predicted chromatographic response surfaces. In these figures, the estimated resolution factors, retention times and peak heights as a function of HBA concentration and pH, methanol content and pH, TBAI concentration and pH and/or TBAI and KHP concentrations are shown. With the use of these visualized three-dimensioned plots, it is possible to determine the optimum mobile phase conditions by restricting the optimization criteria within a required range. After rearranging R_s, t_R and/or h, Figs. 2b–5b show the relevant chromatographic response surfaces and acceptable mobile phase conditions that can be used in the practice.

Obviously, the simultaneous effects of mobile phase variables on the separation and detection of inorganic anions are not as simple as in the case of single-factor effects. This is because both the resolution factor (R_s) and the peak height (h) are not direct functions (or could at least be very complex functions) of the mobile phase variables and, in particular, the interactions between factors can play an important role, as we can see from these figures. Information obtained from these response surfaces may not be easy to obtain with the normally used "one factor at a time" method. Developing a multifactor and simultaneous method for estimating the interactions of the variables in the mobile phase, leading to the optimization of the mobile phase composition, is certainly necessary. In this work, some of the experimental sets we used were not organized in a proper factorial design, which diminishes the quality of the computer predictions and reduces the possibility of finding the optimal conditions for the eluent to achieve the selected criteria: maximum (or minimum) separation of critical peak pairs, reduction in total analysis time, maximum peak heights for the various components, etc. However, the present approach is valuable not only in terms of the visualization of the response surface as a function of two variables using a very limited number of experiments, but also in terms of the methodology, which provides a simple, straightforward way to achieve multifactor mobile phase optimization for the prediction of resolution factor and the prediction of the peak height (or peak area) in a complex inorganic ion separation problem in which both ion-exchange and ion-interaction chromatographic systems were employed.

With the optimization procedure described in this paper, we obtained, in the case of ion-exchange chromatography, baseline separation for eight anions (F^-, CO_3^{2-}, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}) with a total elution time of less than 10 min (Fig. 6). The mobile phase composition for HBA is

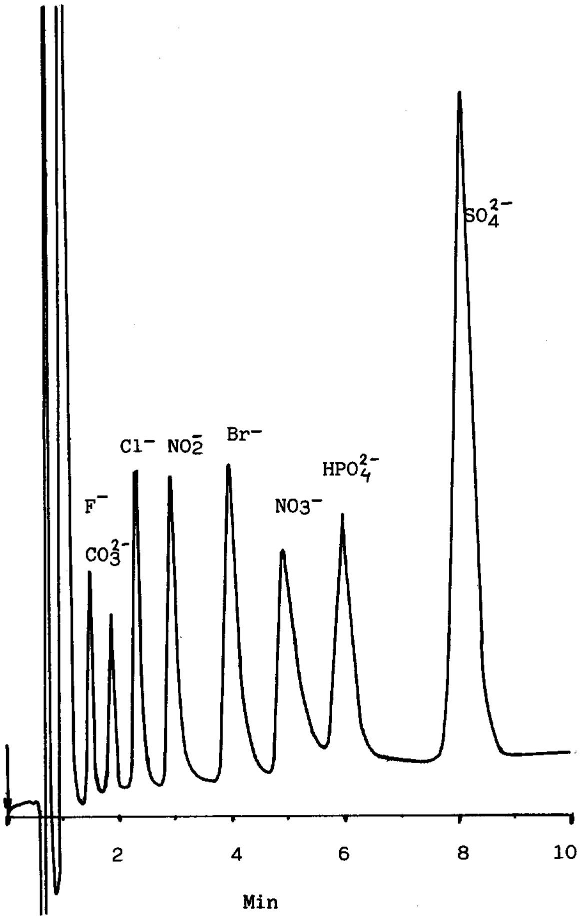


Fig. 6. Separation of eight anions —fluoride, carbonate, chloride, nitrite, bromide, nitrate, phosphate and sulphate— at 3 ml/min and 5 m*M* *p*-hydroxybenzoic acid, 3% methanol, pH 9.5, on a 250 × 3.9 mm I.D. PRP-X100 column. Conductivity detection was used.

5 m*M* and pH is 9.5–9.8 with addition of 3% methanol. In the case of ion-interaction chromatography baseline separation was obtained for five anions (Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}) with a total elution time of less than 9 min. The mobile phase composition for TBAI is 8–8.5 m*M*, for KHP is 1 m*M*, pH of 6–6.5.

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