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REVIEW

COMPARISON OF OPTIMIZATION METHODS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING MIXTURE DESIGNS AND MULTI-CRITERIA DECISION MAKING

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CONTENTS

1. INTRODUCTION

In liquid chromatography, special attention is given to problems of mobile phase optimization. In recent reviews[1–5] attention has been paid to the choice of methods and criteria. Usually the methods are classified according to the mathematical technique used in sequential and simultaneous methods. The criteria can be divided into simple or elemental criteria such as the resolution, the analysis time or the number of peaks and into composite criteria or response functions, which are some function of one or more elemental criteria.

The sequential simplex procedure is a multi-factor method often used with composite criteria to optimize ternary mobile phase systems with regard to separation and analysis time and possibly also for the number of peaks at the same time[3]. The main restrictions of the method are caused by the complexity of the optimization function, which tries to quantify several values for one or more elemental criteria by a single number. This leads to ambiguous results, as the shape of the response surface depends on the *a priori* definition of the composite criterion[6,7]. Moreover, local optima are produced when peak crossover occurs, which the simplex is unable to distinguish from the global optimum[3].

Simultaneous methods are also called regression methods because a model is used to approximate the chromatographic behaviour. Frequently a mixture design is used and the logarithm of the capacity factor of the individual solutes is modelled as

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a function of the mobile phase composition. From the models, the resolution between pairs of peaks can be calculated. If the method is applied to isoeluotropic ternary[8] and quaternary[9] solvent systems, the selectivity of the mobile phase is optimized but the optimization of the analysis time by changing the solvent strength should be impossible, because the mobile phase has a constant solvent strength. Although local optima caused by peak overlap are avoided, a better separation with respect to resolution may be missed, because the full variability of a four-solvent mobile phase system is not exploited[3].

From two gradient elution runs, Quarry *et al.*[10] predicted the capacity factors in binary eluent mixtures and optimized the mobile phase composition by means of a relative resolution map[11]. If the composition of a binary solvent system changes, the solvent strength as well as the selectivity alter, and the band spacing is varied using "solvent strength selectivity"[12–14].

Ternary mobile phases consisting of water and two organic modifiers were optimized by Belinky[15] using a triangular mixture design. Solvent strength and solvent selectivity were varied for the optimization of the resolution. A similar approach is the window diagram technique[16], which reveals the global optimum of a ternary mobile phase system by constructing a minimum resolution or selectivity map. Subsequently the factor space is scanned for the smallest capacity factor of the last component, while maintaining a specified minimum value of the selectivity. This implies that an *a priori* decision with regard to the desired selectivity has to be made.

If in an optimization procedure the solvent strength in addition to the solvent selectivity is varied then both the analysis time and the resolution may be optimized in an isocratic separation. If, however, the range of the capacity factors of the solutes is too large, gradient elution is to be preferred because it provides a faster overall separation. The optimization of binary gradient elutions requires two gradient runs to be carried out according to an approach that relies strongly on the "solvent strength selectivity" concept[17], while Kirkland and Glajch[18,19] optimized multi-solvent gradient separations by running several initial gradients according to a triangular mixture design.

This paper will be confined to the optimization of isocratic separations, for which it has been argued that the combination of solvent strength and solvent selectivity optimization provides better separations[20]. It has been shown that the variation of the water content of binary, ternary and quaternary solvent mixtures alters their selectivity[21] and the tetrahedron design allows the variation of the fraction of water and three organic modifiers of a four-solvent mobile phase system. The global optimum for the resolution can be found[22], but no procedure has been developed to locate the chromatograms that are the best with regard to separation as well as analysis time.

In this paper, a method and software are presented that locate the global optima with respect to separation and analysis time of (binary), ternary and quaternary eluent mixtures and eliminate the necessity to make *a priori* decisions. Solvent strength and solvent selectivity can be varied and the trade-off between resolution and analysis time is quantitatively presented. The necessity for making preliminary assumptions about the relative importance of criteria is eliminated.

The method combines regression techniques with multi-criteria decision making (MCDM)[23] and allows the comparison of the results that can be obtained with

different mobile phase systems *i.e.*, the results of the optimization with isoeluotropic ternary and quaternary mobile phase systems can be compared with those resulting from an optimization of non-isoeluotropic (binary), ternary and quaternary mobile phase systems. Moreover, different criteria can be used to define the quality of the separation.

2. THEORY

2.1. Experimental design and response surface

Multi-criteria decision making cannot be combined with sequential optimization methods. Hence the method is based on the use of mixture designs for the optimization of binary, ternary and quaternary mobile phase systems and starts with modelling the logarithm of the capacity factor, k, as a function of the mobile phase composition. For clarity, the discussion is limited mainly to the use of ternary and quaternary systems.

The factor space of a quaternary eluent consisting of water, methanol, acetonitrile (ACN) and tetrahydrofuran (THF) can be represented by a tetrahedron at the vertices of which the pure solvents are located. If water (component x_1) is placed at the top of the tetrahedron and the three organic modifiers (x_2, x_3, x_4) at the vertices of the ground surface, then the solvent strength of the mobile phase systems decreases in the direction of the top of the tetrahedron as the fraction of water of the system increases. The edges and faces of the tetrahedron are the factor spaces for the binary and non-isoeluotropic ternary eluents, respectively. The factor space of an isoeluotropic quaternary system is represented by a transversal triangular cross-section through the tetrahedron and isoeluotropic ternary systems are found on the sides of this triangle (Fig. 1).

The location and the number of design points are to be determined after the selction of the factor space, the latter being a line for an isoeluotropic ternary mobile phase system, a triangle corresponding with a face of the tetrahedron for a non-isoeluotropic ternary system, a triangular cross-section for an isoeluotropic quaternary mobile phase system and the tetrahedron for a quaternary system. The design points have to be distributed evenly in the design space, which is found by constraining the factor space.

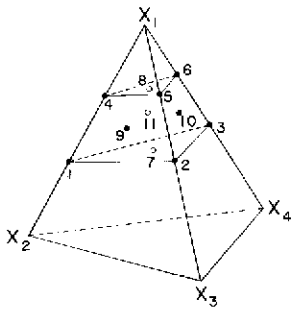

Fig. 1. Mixture tetrahedron of quaternary mobile phase system with constrained design; x_1 = water, x_2 = methanol, x_3 = ACN, x_4 = THF. The solvent strength of the "upper" cross-section, defined by points 4, 5 and 6, equals 1.0; the solvent strength of the "lower" cross-section, defined by points 1, 2 and 3, equals 1.7. Only eleven design points of the seventeen in Table 2 are shown.

For the non-isoeluotropic eluents, two constraints on the solvent strength are needed: an "upper" constraint at low solvent strength in order to obtain a capacity factor of the last peak smaller than a given acceptable value, *e.g.*, 20, and a "lower" constraint at high solvent strength that confines the capacity factor of the first peak to values greater than 1 (Fig. 1).

The design space of isoeluotropic eluent systems is found by constraining the factor space in such a way that the solvent strength is constant and the capacity factors of all solutes are smaller than a given value, *e.g.*, 10. The appropriate solvent strength of the mixtures can be determined by a gradient run in one binary system[10] and the use of transfer rules for the solvent strength to find the corresponding compositions of the two other binary systems[24].

The number of the design points that is required depends on the dimensions of the factor space and the order of the model that is used[25]. Although cubic and special cubic models can be selected, this discussion will be limited to the use of linear and quadratic models. The quadratic equation for one solute in a quaternary system is

$$\ln k = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{14}x_1x_4 + \\ + a_{23}x_2x_3 + a_{24}x_2x_4 + a_{34}x_3x_4 + E \quad (1)$$

where x_1, x_2, x_3 and x_4 are fractions of the components water, methanol, ACN and THF, respectively, a_1–a_{34} are the coefficients to be estimated and E is the residual error. For the estimation of the coefficients and E we need in this instance at least eleven experiments. For a simpler system, a non-isoeluotropic ternary eluent for example, and a simpler model, say a linear model, at least four experiments are required. The number of required experiments is determined by the necessity to estimate the coefficients a_1, a_2, a_3 and E. The number of components or binary pseudo-components that are used and the order of the model determine the number of terms in the model equation, *i.e.*, for a ternary mixture and a quadratic model the equation can be found by deleting from eqn. 1 all terms containing x_4. In that event at least seven design points are required. For a ternary mixture and a linear model the interaction terms x_ix_j can also be omitted and at least four experiments are necessary.

This number of experiments has to be performed simultaneously and in a random order. In this sense the method has to be classified as a simultaneous method. The possibility is offered, however, of starting with a two- or three-dimensional linear or quadratic model and of progressing to four-component mobile phases and more complex models by performing the additional experiments required, guided by the results of statistical model evaluation and the adequacy of the predicted separation.

2.2. Criteria

As the proposed procedure is intended in the first place for samples with a known number of components, this number is not included as a quality criterion. Further, the criteria have to be selected in accordance with the last step of the optimization procedure, *i.e.*, MCDM.

The essence of MCDM is to judge the different quality aspects of a chromatogram individually and quantitatively. Accordingly, the criteria have to define unequivocally one quality aspect. In the MCDM procedure, criteria values are

weighed continuously against each other. Therefore, complex separation criteria and criteria which have a threshold character or a discontinuity with respect to the resolution are excluded, because the resolution, as given by the expression

$$R_{s_{1,2}} = N^{0.5}(k_2 - k_1)/2(k_2 + k_1 + 2) \tag{2}$$

increases continuously with the separation of two peaks. Hence the resolution and the selectivity factor, alpha, are incorporated, but simple criteria such as the valley-to-peak ratio and the peak separation are not included, because of their threshold character (see Fig. 2 in ref. 6). The resolution and the selectivity factor are calculated from the predicted capacity factors at all eluent compositions necessary to obtain a grid with a 1% interval in the fractions x_i. The resolution and alpha are calculated for every pair of peaks, but only the minimum values are stored and plotted at the grid points and a minimum resolution or minimum selectivity plot is produced.

A disadvantage of using alpha is that the influence of the capacity factor on the resolution is not taken into account, as the resolution improves strongly for values of the capacity factor up to about 5, whereas the value of alpha remains constant. Nevertheless alpha has been incorporated because it does not depend on the column dimensions and because a comparison with other separation criteria was desirable.

It has been argued that the minimum resolution (and minimum alpha) plots have the disadvantage that only the worst separated pair of peaks is considered and the remaining part of the chromatogram is not considered[4]. Although this argument is of questionable value when MCDM is applied, the calibrated normalized resolution product, CNRP[7], is also considered and can be plotted at every grid point:

$$\text{CNRP} = \prod_{i}^{n} (S_{i,j}/S^*) \tag{3}$$

where $S^* = (1/n)\sum_{i}^{n} S_{i,j-1}$ is the average separation factor for all pairs of peaks, including a hypothetical peak at $t = t_0$; $S_{i,j}$ is the separation factor for two components i and j and equals $2R_{s_{i,j}}$ for $N = 1$ (n = number of solutes, N = plate number). The CNRP aims at an even spacing of all peaks throughout the chromatogram, beginning with a hypothetical peak at $k = 0$. It has the disadvantage of being ambiguous, *i.e.*, a set of chromatograms corresponds to a single value of the criterion.

The resolution, alpha and the CNRP are all separation criteria, which should be weighed against a criterion that assesses a different quality aspect of the chromatogram. The analysis time was selected as the second quality aspect of the chromatogram and the corresponding criterion is the capacity factor of the last peak. The capacity factor of the last peak is also predicted from the models at every grid point of mobile phase compositions and plotted or stored for later use.

2.3. Multi-criteria decision making

In the previous section, the quality of the chromatogram was defined by two kinds of criteria, one for the separation aspect and one for the analysis time. We select

the minimum resolution, Min Res, and the analysis time of the last peak, Max k, for the explanation of the MCDM procedure[23]. In the design space both criteria are predicted at all eluent compositions necessary to obtain a grid with a 1% interval in the fractions x_i. Hence every eluent composition is characterized by one value of the Min Res and one value of the Max k.

To achieve an easy and quantitative weighting of both criteria against each other, an MCDM diagram is constructed, consisting of two perpendicular coordinate axes. On the horizontal axis, called the time axis a scale for Max k is constructed and on the vertical axis a scale of Min Res values is given. Now, every mobile phase composition of the grid can be represented by a point in the diagram, defined by one value of the Min Res and one value of Max k. This results in a cloud of points, of which only the "pareto optimal" (PO) points are depicted in the diagram. A point is called pareto optimal if there exists no other point in the diagram, *i.e.*, eluent composition, that yields an improvement in one criterion without causing a deterioration of the other criterion. The PO points constitute the upper left border of the cloud of points representing all eluent compositions, and only the PO points are depicted in the MCDM diagram; all other points represent inferior combinations of the two criteria and are not shown (see Fig. 4, open circles).

Only the PO points give the best possible combinations of the two criteria. They show the pay-off between both criteria for a given mobile phase system and characterize the performance of the eluent by a string of points[26].

3. EXPERIMENTAL

Experimental details with regard to the test solutes used, the quality of the solvents and the chromatographic apparatus and procedures have been given elsewhere[21]. Calculations were performed on an IBM-XT compatible personal computer using the POEM software package written in Pascal.

The POEM (Predicting Optimal Eluent Mixtures) package is designed for the optimization of mobile phases consisting of two, three or four (pseudo)components using mixture designs in the separation of at most fifteen solutes. It is an interactive menu-driven program containing five main sections. The first two sections handle the definition of the experimental design (numbers of observations, solvents and solutes) and the input of the capacity factors measured at the design points.

The "model" section permits one to choose between different polynomials to model the (logarithm of the) capacity factor as a function of the eluent composition: linear, quadratic, special cubic and cubic mixture models for two, three or four (pseudo)components can be selected. The coefficients of the models are calculated by multiple regression and validated by an analysis-of-variance (ANOVA) table for a linear regression model, by the multiple coefficient of determination, R, and the adjusted R^2 coefficient, which characterize the descriptive capability of the model. Also, an indication of the predictive power of the model is given by the PRESS (predicted error sum of squares) value, which is based on the "leave one out" (LOO) method.

The LOO method is a cross-validation method[27]. In this procedure, different subsets of the dataset are used successively as test sets to validate regression models constructed from the remaining data consisting of the data set minus the test set. In the

LOO method, the test set consists of only one measurement. Therefore, a model is constructed after deleting one observation of the data, then this observation is predicted by a model based on the remaining data and the squared difference between the left-out observation and its prediction is calculated. This procedure can be repeated N times, each time with a different observation deleted from the data (N = the number of observations). The PRESS value is equal to the sum of the squared differences. A mean PRESS value is calculated by dividing the PRESS value by N.

From the models of the capacity factor response surfaces for the Min Res (eqn. 2), the Min Alpha, the CNRP (eqn. 3) or Max k are calculated. It is possible to calculate the response surface for a grid with a 1, 2, 5 or 10% interval in the fraction x_i of each of the (pseudo)components. Also, subspaces of the chosen factor space, *e.g.*, a triangular cross-section or a triangular face of the tetrahedron, can be defined for which the corresponding response surface is calculated. The response surfaces are plotted as contour plots (see Figs. 2A–D, 5, 8–10). The response values and the corresponding eluent compositions can also be listed.

The MCDM section selects the PO eluent compositions for the best combinations of a separation criterion (Min Res, Min Alpha or CNRP) and analysis time (Max k). The PO eluent compositions can be listed and plotted in the design space defined by the selected (pseudo)components (see Figs. 3, 6 and 11). Moreover, an MCDM diagram of the PO points can be plotted for an easy evaluation of the trade-off between separation and analysis time and for the comparison of the performance of different mixtures of solvents (see Figs. 4, 7 and 12).

A chromatogram can be simulated for a given mobile phase composition, plate number and dead time, which are defined by the user (see Fig. 13A).

4. RESULTS

The comparison of the results of optimization procedures that use different factor spaces was the purpose of this investigation, *i.e.*, isoeluotropic ternary, quaternary and non-isoeluotropic ternary and quaternary mobile phase systems were compared. Moreover, the effects of different criteria were studied.

The dependence of the capacity factor on the solvent composition generally increases with increasing molecular size of the solutes[10,28], hence test solutes were chosen that do not differ substantially in molecular size but constitute a group of small molecules of different functionality (Table I). The design space chosen is a truncated tetrahedron (Fig. 1), because the full variability of a quaternary system was to be investigated. The lower constraint is the cross-section defined by the points 1, 2 and 3 and has a solvent strength of approximately 1.7. The solvent strength was calculated

TABLE I

SOLUTES OF THE TEST SAMPLE

No.	*Component*	*No.*	*Component*
1	BN = benzonitrile	4	PE = 2-phenylethanol
2	BA = benzaldehyde	5	TL = toluene
3	NB = nitrobenzene	6	pC = *p*-cresol

TABLE 2

MOBILE PHASE COMPOSITIONS AT THE DESIGN POINTS AND MEASURED CAPACITY FACTORS OF THE SOLUTES

Point No.	*Fraction of*				*Capacity factors of the solutes*[a]					
	Water	*CH_3OH*	*ACN*	*THF*	*BN*	*BA*	*NB*	*PE*	*TL*	*pC*
1	0.350	0.650	0.000	0.000	0.867	0.737	1.190	0.683	1.833	0.713
2	0.485	0.000	0.515	0.000	1.380	1.173	1.676	0.705	2.584	0.971
3	0.614	0.000	0.000	0.386	2.011	1.619	3.102	1.159	5.460	2.534
4	0.600	0.400	0.000	0.000	3.127	2.595	4.244	2.234	8.995	2.624
5	0.685	0.000	0.315	0.000	3.869	2.940	5.366	1.831	10.011	2.705
6	0.765	0.000	0.000	0.235	5.848	4.043	11.598	3.283	23.484	9.266
7	0.466	0.221	0.173	0.140	0.919	0.780	1.355	0.624	2.306	0.935
8	0.680	0.135	0.105	0.080	3.480	2.641	5.677	2.237	11.934	4.030
9	0.440	0.280	0.280	0.000	0.928	0.773	1.201	0.557	1.979	0.680
10	0.580	0.000	0.210	0.210	1.617	1.328	2.289	0.906	4.006	1.678
11	0.530	0.235	0.000	0.235	1.305	1.058	2.337	0.905	3.963	1.658
12	0.390	0.488	0.061	0.061	0.695	0.536	1.056	0.526	1.706	0.695
13	0.500	0.050	0.400	0.050	1.294	1.102	1.667	0.718	2.751	1.011
14	0.580	0.042	0.042	0.336	1.739	1.402	2.741	1.051	4.888	2.130
15	0.610	0.312	0.039	0.039	2.542	2.057	4.061	1.863	8.580	2.854
16	0.700	0.030	0.240	0.030	4.051	3.041	5.938	2.087	11.856	3.374
17	0.730	0.027	0.027	0.216	4.786	3.402	9.088	2.788	18.200	7.089

[a] For abbreviations, see Table 1.

for the binary mixture of water and methanol ($x_1 = 0.35$; $x_2 = 0.65$), in which the capacity factor of the fastest eluting solute is slightly smaller than 1 and Max $k = 1.8$. Subsequently, the compositions of binary mixtures of water with ACN and THF were calculated that correspond to a solvent strength of 1.7[29]. The upper constraint of the design space is the cross-section defined by the points 4, 5 and 6 and has a solvent strength of 1.0. The capacity factor of the last-eluting solute was approximately 9 in the binary mixture of water and methanol ($x_1 = 0.60$; $x_2 = 0.40$), which was used for the calculation of the compositions of the binary mixtures of water with ACN or THF.

The capacity factors of the test solutes were measured at seventeen mobile phase compositions in the design space. For simplicity, only eleven design points are shown in Fig. 1. Although eleven design points suffice for the construction of a four-factor quadratic model and, although we showed previously[21] that on the basis of these measurements an accurate prediction of the capacity factors of the test solutes was possible, we decided to add six more points in order to have more degrees of freedom for the statistical evaluation of the models and to obtain a better estimate of the model coefficients. The measured capacity factors and the corresponding mobile phase compositions are given in Table 2.

Averaged over the six test solutes, the linear model explained 97.46% of the variation in the data and the relative standard deviation of the differences between the measured and the predicted capacity factors (12 degrees of freedom) was 13.0%. The quadratic model explained on average 99.65% of the variation in the data and the relative standard deviation of the residuals was 6.2% (6 degrees of freedom). Although

an increase of 2.19% in the explained variation by replacing a linear by a quadratic model may seem a small improvement, the quadratic model is certainly to be preferred, because this small increase more than halves the relative standard deviation between the predicted and the measured capacity factor, *i.e.*, from 13.0 to 6.2%.

Averaged over the six solutes, the relative standard deviation of the special cubic model was 4.0%, but the average mean PRESS value of the special cubic model was about 1.9 times larger than the corresponding value of the quadratic model. This indicates that the quadratic model is superior to the special cubic model with respect to predictive power. Therefore, the quadratic model was used for the comparison of the different optimization procedures.

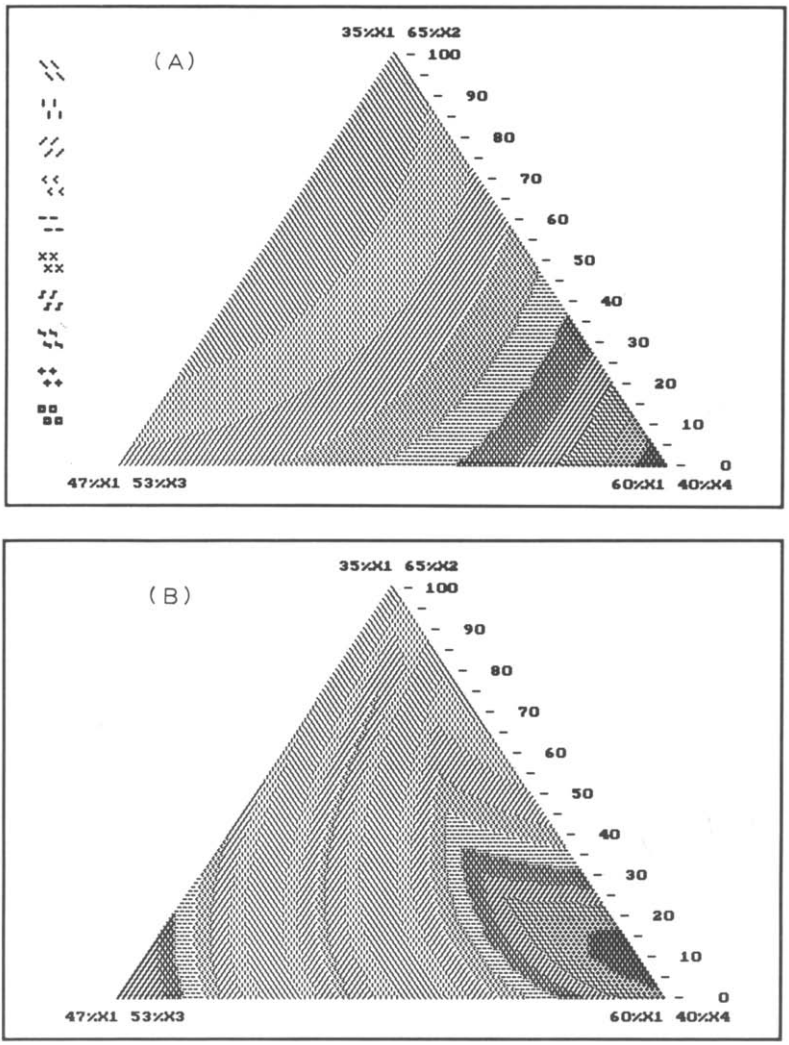

Fig. 2.

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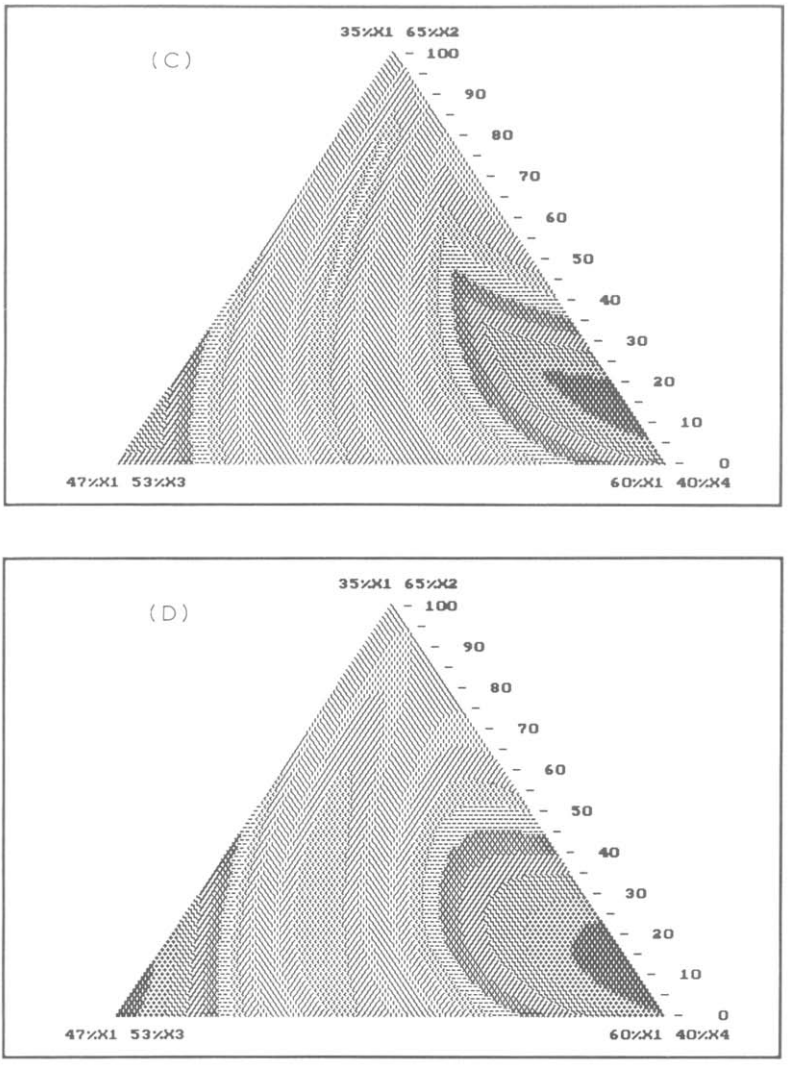

Fig. 2. (A) Response surface of the capacity factor of the last component of Max k of the isoeluotropic quaternary system with solvent strength 1.7, defined by the points 1, 2 and 3 of the lower triangular cross-section in Fig. 1. Different symbols correspond to ten different ranges of values of the Max k (= contour plot). The lowest range is indicated by backslashes and the highest range by squares. x_1 = Water; x_2 = methanol; x_3 = ACN; x_4 = THF. Composition of the pseudo-components is reported near the corresponding vertex. (B) Response surface of the Min Res of the same mobile phase system as in A. (C) Response surface of the Min Alpha of the same mobile phase system as in A. (D) Response surface of the CNRP of the same mobile phase system as in A.

4.1. Isoeluotropic ternary and quaternary mobile phases

In Fig. 2, the response surfaces of the capacity factor of the last peak (Max k), of the minimum resolution (Min Res), of the minimum selectivity factor (Min Alpha) and of the CNRP are shown for the lower triangular cross-section of tetrahedron. This is

the factor space of the isoeluotropic quaternary mobile phase system with solvent strength 1.7. The factor spaces of the three isoeluotropic ternary mobile phase systems with the same solvent strength are represented by the sides of this triangle.

Fig. 2A shows that the analysis time decreases from the highest value of Max k of 5.1 (Table 3) near the binary pseudo-component water–THF in the direction of the opposite side of the triangle to values in the range 1.6–2.6. This means that the solvent strength transfer rule for THF allows a large variation of the capacity factor of the last solute, which was always toluene in this sample.

The resemblance between the Min Res plot (Fig. 2B) and the Min Alpha plot (Fig. 2C) is striking. Although the two plots differ in detail, the maximum values, (Table 3) are found in a ternary composition consisting of water, methanol and THF, *i.e.*, on the right-hand side of the triangle adjacent to the dark area.

The centre of the dark area of highest values of the Min Res lies around the 10% coordinate of the water–methanol pseudo-component, while the centre of the corresponding area of Min Alpha is found around the 15% coordinate. This is a shift in the direction of lower values of the capacity factors (see Fig. 2A), perceptible throughout the contour plot, which shows that the Min Res criterion, in contrast to the Min Alpha, increases as the capacity factors increase between 1 and 5. The Min Res and the Min Alpha plots indicate peak crossover in the same areas of the factor space (Fig. 2B and C, backslashes). The CNRP plot (Fig. 2D) has an overall resemblance to both of the other response surfaces (Fig. 2B and C). All contour lines are rounded and the maximum area encompasses the maximum areas of both of the previous plots.

The differences between the various plots are evaluated more easily by the comparison of the plots of the PO eluent compositions (Fig. 3) and the corresponding MCDM diagrams (Fig. 4). The PO eluent compositions give the best possible combinations of analysis time (Max k) and a separation criterion (Min Res, Min Alpha or CNRP). Because of the resemblance between the plots of Max k with the Min Res, the Min Alpha and the CNRP, respectively, only the PO plot of the Max k and the Min

TABLE 3

COMPARISON OF PERFORMANCE OF MOBILE PHASE SYSTEMS

Type	*Mobile phase system*	*Max k range*	*Maximum criterion values*			
			Max k[a]	*Min Res*	*Min Alpha*	*CNRP*
Isoeluotropic	Quaternary (1,2,3)[b]	1.6–5.1	4.7	2.5	1.24	0.27
	Quaternary (4,5,6)	9.0–25.4	18.0	3.7	1.28	0.24
	Ternary H_2O–CH_3OH–ACN (4,5)	9.0–10.5	9.1	1.3	1.10	0.09
	Ternary H_2O ACN THF (5,6)	10.5 25.4	18.0	3.7	1.28	0.24
	Ternary H_2O–THF–CH_3OH (4,6)	9.1–25.4	25.4	2.7	1.19	0.18
Non-isoeluotropic	Quaternary (1,2,3,6,4,5)	1.5 25.4	12.0	4.0	1.32	0.31
	Ternary H_2O CH_3OH–ACN (1,2,5,4)	1.5–10.5	4.0	2.2	1.20	0.29
	Ternary H_2O–ACN–THF (2,3,6,5)	2.4–25.4	12.0	4.0	1.32	0.31
	Ternary H_2O–THF–CH_3OH (1,3,6,4)	1.6–25.4	11.7	3.6	1.28	0.31

[a] Gives the analysis time in Max k necessary to obtain the maximum Min Res.

[b] Indicates the factor space as given by the points in Fig. 1.

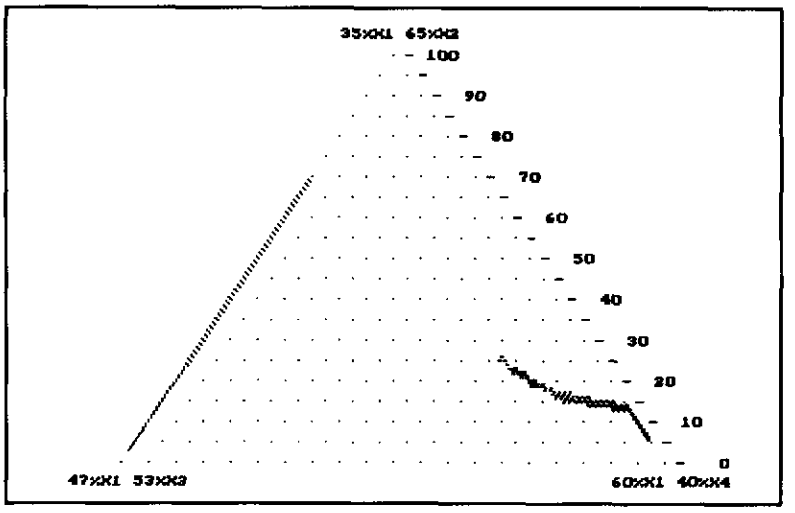

Fig. 3. Plot of the PO eluent compositions giving the best combinations of the Min Res and the Max k of the same mobile phase system as in Fig. 2.

Res is shown (Fig. 3). Compared with the PO plot of the Min Res, the PO plot of the Min Alpha misses a number of ternary solvent composition in the lower left-hand corner of the plot and the PO plot of the CNRP misses most quaternary eluent compositions.

To evaluate the importance of these missing eluent compositions, one needs also the MCDM diagrams (Fig. 4), which show the trade-off between separation and analysis time. The supplementary lists (not given) that state the eluent compositions of the PO points in the MCDM diagrams show that the discontinuities in the MCDM diagrams correspond to the above-mentioned missing eluent compositions. In the

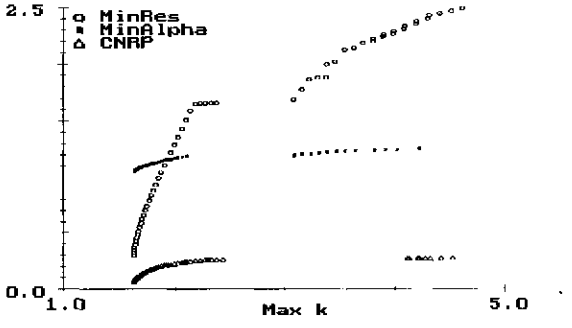

Fig. 4. MCDM diagrams. Points represent the PO eluent compositions of the same mobile phase system as in Fig. 2. The upper curve (○) represents the eluent compositions giving the best combinations of the Min Res and the Max k (Fig. 3); the middle curve (■) represents the eluent compositions giving the best combinations of the Min Alpha and the Max k; the lower curve (△) represents the eluent compositions giving the best combinations of the CNRP and the Max k.

MCDM diagram of Min Alpha and Max k (Fig. 4, solid squares), a Min Alpha value of 1.19 corresponds to a Max k value of 2.1. To improve the separation by a higher value of the Min Alpha, the analysis time must be increased to a Max k value of at least 3.0, because of the discontinuity from 2.1 to 3.0 in the MCDM diagram of the Min Alpha.

The MCDM diagram of the Min Res (Fig. 4, open circles) shows a similar discontinuity for Max k values from 2.3 to 3.0. Both diagrams show that further improvement in the separation of the worst separated pair of peaks is possible only for analysis times greater than a Max k value of 3. This indicates that the Min Res and the Min Alpha are approximately equivalent criteria.

The CNRP expresses the separation between all pairs of peaks by a number from zero to one; also, it promotes an even spreading of the peaks throughout the chromatogram and favours shorter analysis times. The MCDM diagram of the CNRP and Max k (Fig. 4, open triangles) reveals an improvement in the separation quality up to a CNRP value of 0.26 at a Max k value of 2.4. A further small increase in the CNRP to 0.27 necessitates Max k values greater than 4.3. In the range of Max k values from 3.0 to 4.3 the CNRP detects no improvement in the quality of the chromatogram, but in this range of Max k the separation of the worst separated pair of peaks, as indicated by the Min Res, increases from 1.7 to 2.4, which is an important improvement. This difference between the MCDM diagrams of the Min Res and the CNRP is reflected by the corresponding PO plots. In comparison with the PO plot of the Min Res (Fig. 3), the PO plot of the CNRP misses most quaternary eluent compositions. For these missing eluent compositions the CNRP fails to detect an improvement. The conclusion has to be drawn that an ambiguous criterion such as the CNRP is inferior to simple criteria such as Min Res and Min Alpha when MCDM is applied.

When the response surfaces of the upper triangular cross-section, the factor

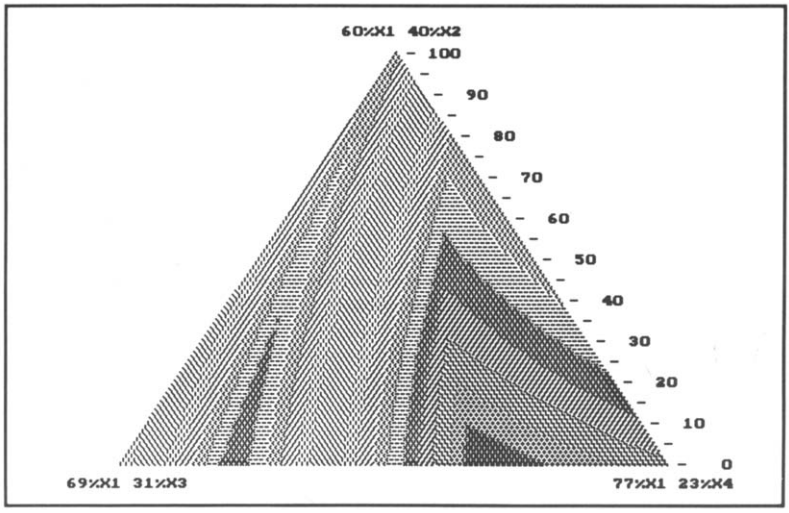

Fig. 5. Response surface of the Min Res of the isocluotropic quaternary system with solvent strength 1.0 defined by the points 4, 5 and 6 of the upper triangular cross-section of Fig. 1. x_1 = Water; x_2 = methanol; x_3 = ACN; x_4 = THF. Composition of the pseudo-components is reported near the corresponding vertex.

space of the isoeluotropic quaternary and ternary mixtures with a solvent strength value of 1.0, are considered, the Max k plot (not shown) again shows a decrease in the analysis time from Max k values from about 25.4 to 9.0 in the same direction as in Fig. 2A. The Min Res plot (Fig. 5) and the Min Alpha plot (not shown) have the same shape, but differ from the corresponding response surfaces at the higher solvent strength of 1.7, depicted by Fig. 2B and C. The maximum values for the Min Res and the Min Alpha (Table 3) are both found at a ternary system in the dark area adjacent to the base of the triangle (Fig. 5). Comparison of the response surfaces of the Min Alpha at high solvent strength (1.7) (Fig. 2B) and at low solvent strength (1.0) (similar to Fig. 5) shows that variation of the solvent strength alters the shape of the response surface or, stated differently, not only the solvent strength but also the selectivity of the system is changed.

Because the capacity factors of the worst separated pair of peaks are greater than about 9, the response surface of the Min Alpha (not shown), the plot of PO eluent compositions (not shown) and the corresponding MCDM diagram of the Min Alpha and Max k (Fig. 7, solid squares) have shapes very similar to the corresponding figures for the Min Res (Figs. 5, 6 and 7, open circles). The response surface of the CNRP (not shown) again has an overall but slight resemblance with Fig. 5 and the contour lines are curved. The plot of the PO eluent compositions of the CNRP and Max k differs from the corresponding plots of the Min Res and the Min Alpha. The MCDM diagram of CNRP *vs.* Max k (Fig. 7, open triangles) has a larger discontinuity of 2 k units around the Max k value of 13. A discontinuity in the MCDM diagram indicates that an increase in analysis time, equal to the length of the discontinuity, is necessary in order to obtain a small gain in the quality of the separation. Unnecessary large discontinuities are undesirable. This supports our previous conclusion that the response surface generated by the CNRP is inferior to the Min Res in this context and the CNRP is no longer considered.

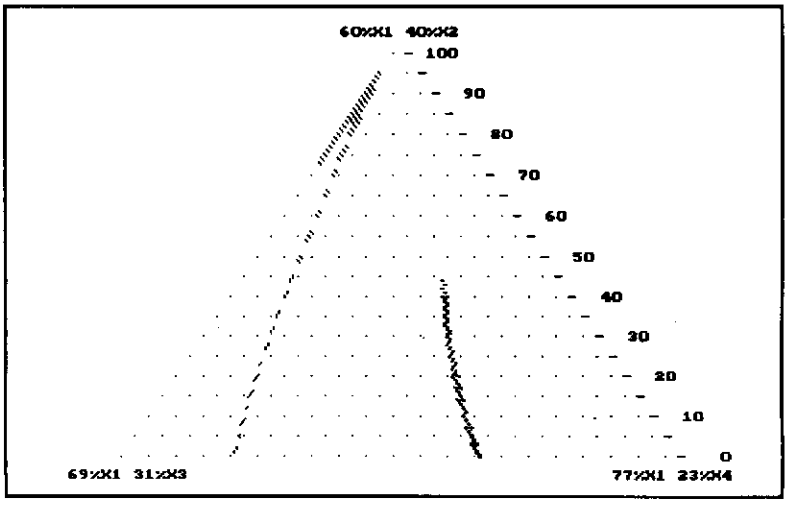

Fig. 6. Plot of the PO eluent compositions giving the best combinations of the Min Res and the Max k of the same mobile phase system as in Fig. 5.

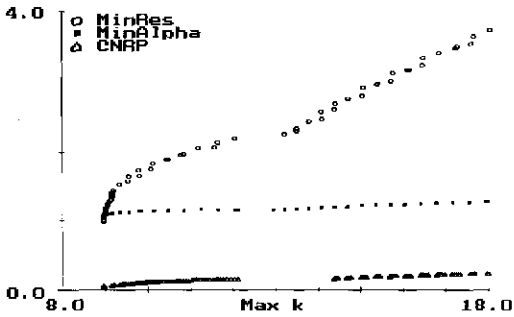

Fig. 7. MCDM diagrams. Points represent the PO eluent compositions of the same mobile phase system as in Fig. 5. The upper curve (○) represents the eluent compositions giving the best combinations of the Min Res and the Max k (Fig. 6); the middle curve (■) represents the eluent compositions giving the best combinations of the Min Alpha and the Max k; the lower curve (△) represents the eluent compositions giving the best combinations of the CNRP and the Max k.

The MCDM diagrams of the Min Res and the Max k of the quaternary mobile phase system of solvent strength 1.7 (Fig. 4) and of the system of solvent strength 1.0 (Fig. 7) demonstrate clearly that alteration of the solvent strength has severe consequences with regard to the optimization of this sample. At high solvent strength a Min Res of 1.5 can be obtained in an analysis time of Max k values slightly smaller than 2, whereas in the lower solvent strength system the same Min Res requires a Max k value of about 9. The maximum Min Res at solvent strength 1.7 is about 68% of the maximum value at solvent strength 1.0, but the Min Alpha differs by only 3% in the two systems (Table 3). Hence higher resolutions are obtained at the cost of longer analysis times and the shortest analysis time that results in a satisfactory resolution cannot be realized in the system with low solvent strength.

The above discussion has been devoted entirely to isoeluotropic quaternary systems, because the optimization of quaternary systems is methodologically superior to that of three isoeluotropic ternary systems. In fact, the three isoeluotropic ternary systems constitute three different factor spaces, *i.e.*, the sides of the isoeluotropic quaternary triangle, which have to be investigated by the construction of three different models. The construction of a quadratic model for an isoeluotropic ternary system consisting of two binary pseudo-components requires four measurements to determine the coefficients a_1, a_2 and a_{12} and the E term of the model equation. Hence for three isoeluotropic systems twelve design points should be measured, but because the three systems share the same three pseudo-components only nine measurements are necessary. Even if a statistical evaluation of the models is omitted and the E term is not determined, six measurements are still necessary. Seven measurements, or six if the residuals (E term) are not estimated, suffice for the construction of a quadratic model for an isoeluotropic quaternary system consisting of three pseudo-components. In the latter instance, however, the response surface of the whole triangular factor space can be modelled, which means that information is obtained on about 5000 solvent compositions of a 1% grid, whereas the ternary systems produce information on only 300 solvent compositions for the same experimental effort. The same line of reasoning applies if linear models are used[2], which are generally inferior to quadratic models, as was verified again in the present case (see above).

Figs. 5 and 6 illustrate the consequences of the limited factor space of the isoeluotropic ternary systems. Although the Min Res of all three ternary systems is sufficient, *i.e.*, a Min Res value of 1.3 can be reached (Fig. 5, Table 3), only a few of the PO eluent compositions of the isoeluotropic quaternary system are realized. In the ternary system water–methanol–ACN, represented by the left-hand side of the triangle in Fig. 6, PO points are found near the apex. The maximum Min Res is about 1.3 (Max $k = 9.1$; Table 3). The only other PO composition is found in the ternary system water ACN THF, the base of the triangle where a Min Res of 3.7 can be reached (Max $k = 18.0$). This does not imply that in the ternary systems no minimum resolutions between 1.3 and 3.7 can be realized, but the ternary eluents which can produce these values of the Min Res are inferior to the quaternary eluent systems that produce these values for the Min Res in shorter analysis times (Fig. 6).

4.2. Non-isoeluotropic ternary and quaternary mobile phases

The contour plots of the Min Res (Fig. 8) and of the Min Alpha (not shown) for the non-isoeluotropic ternary mobile phase system water–methanol–ACN are very similar. The difference stems again from the fact that at low values of the capacity factor higher values of alpha are needed to obtain the same resolution. As a consequence, the contours of the Min Alpha plot are drawn out to the direction of smaller capacity factors as compared with the Min Res plot. This applies also to the two other ternary systems, water–ACN–THF and water–THF–methanol, of which the Min Res response surfaces are depicted in Figs. 9 and 10. It is remarkable that the region of maximum Min Res and Min Alpha is found at intermediate values of the solvent strength (Figs. 8–10). This implies that the selectivity of ternary mobile phase systems is changed by variation of the water content, and that water is not to be considered as a "carrier" that influences only the solvent strength.

From the response surfaces one can obtain an impression of which eluent

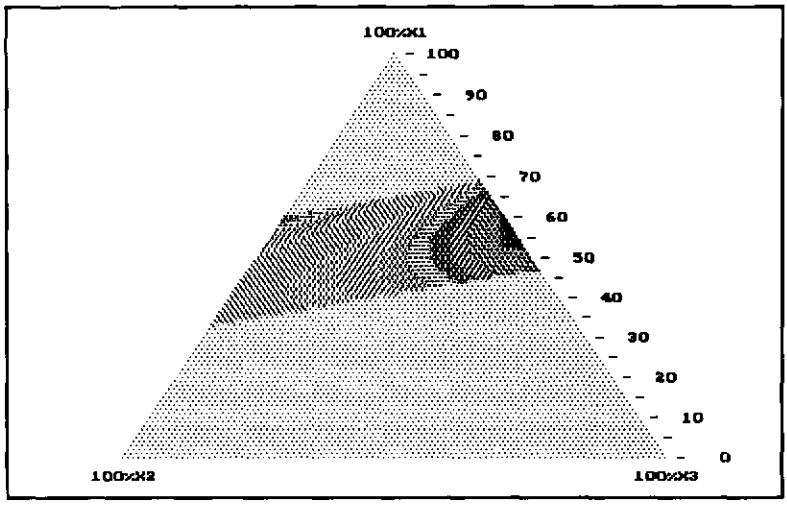

Fig. 8. Response surface of the Min Res of the non-isoeluotropic ternary system defined by the points 1, 2, 5 and 4 of a triangular face of Fig. 1. x_1 = Water, x_2 = methanol, x_3 = ACN.

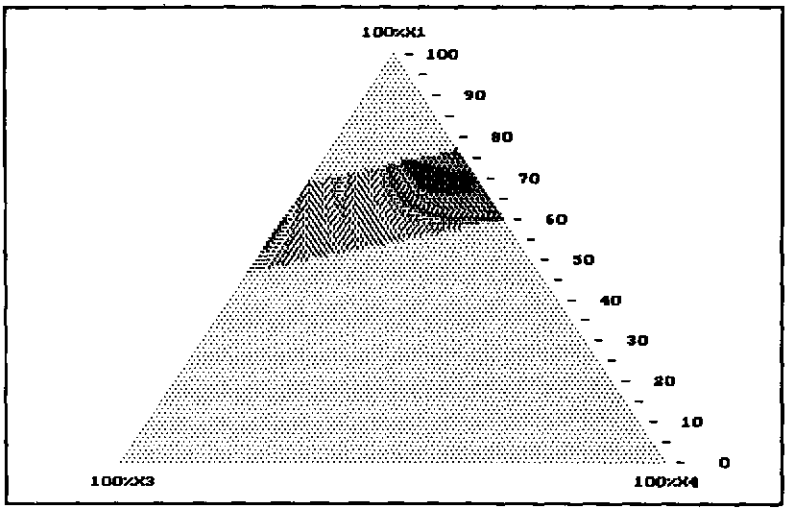

Fig. 9. Response surface of the Min Res of the non-isoeluotropic ternary system defined by the points 2, 3, 6 and 5 of a triangular face of Fig. 1. x_1 = Water, x_3 = ACN, x_4 = THF.

compositions will produce chromatograms with the shortest analysis time for a given value of the Min Res. These compositions are found in the "lower" part of the region that indicates this value of the Min Res in the contour plot, because the solvent strength increases from the apex to the base of the triangle. This is corroborated by the pattern of plots of PO eluent compositions of the three ternary systems. Fig. 11 shows the PO plot for the water–methanol–ACN system, in which the points of PO composition approach the region of maximum Min Res from the side of the high

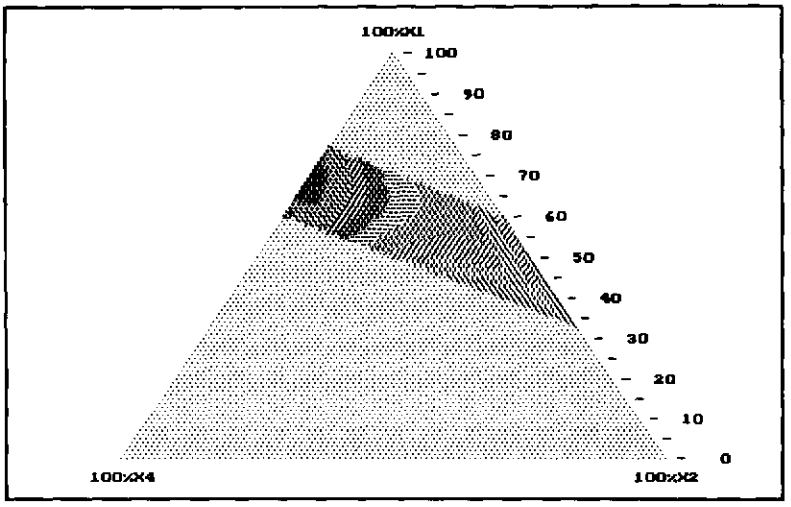

Fig. 10. Response surface of the Min Res of the non-isoeluotropic ternary system defined by the points 1, 3, 6 and 4 of a triangular face of Fig. 1. x_1 = Water, x_2 = methanol, x_4 = THF.

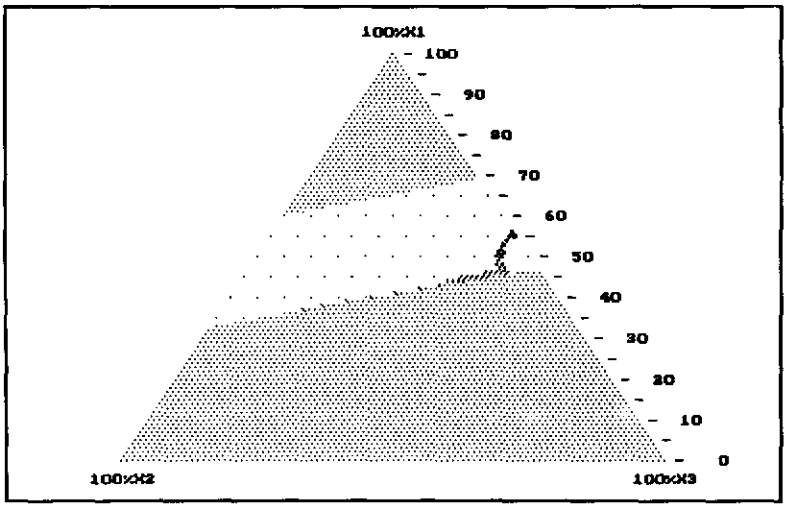

Fig. 11. Plot of the PO eluent compositions giving the best combinations of the Min Res and the Max *k* for the mobile phase system water–methanol–ACN (Fig. 8).

solvent strength. As mentioned above, the solvent strength of the "lower" constraint of the design space is 1.7 and the solvent strength of the "upper" constraint is 1.0.

The MCDM diagrams of the three ternary systems (Fig. 12) show the difference in performance with respect to resolution and analysis time of these non-isoeluotropic ternary systems. Mobile phases consisting of water, methanol and ACN give the shortest analysis times up to a Min Res value 2.2 (Fig. 12, open circles). The water–ACN–THF system (Fig. 12, backslashes) performs equally well for Min Res values between 1.7 and 2.2, *i.e.*, up to a Max *k* value of 4.0. Higher values of the Min Res can be obtained with the water–methanol–THF system (Fig. 12, dashes) at the cost of longer analysis times. From a Min Res value of about 2.7 the water–ACN–THF performs equally well (Table 3).

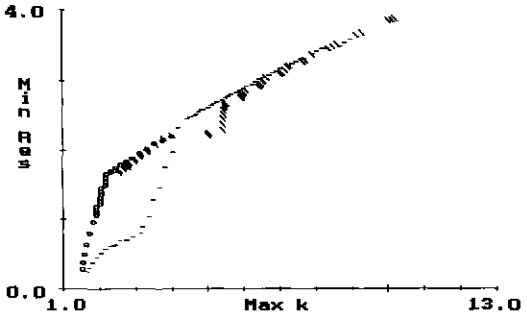

Fig. 12. MCDM diagrams. Points represent the PO eluent compositions giving the best combinations of the Min Res and the Max *k*. Open circles, mobile phase system water–methanol–ACN (Fig. 8); backslashes, mobile phase system water–ACN–THF (Fig. 9); dashes, mobile phase system water–THF–methanol (Fig. 10).

If the performance of the three ternary systems is compared with those of the two isoeluotropic quaternary systems (Figs. 4 and 7), one can conclude that two of the three ternary systems (water methanol ACN and water ACN–THF) can realize a Min Res of about 1.5 in the same analysis times (Max $k = 2$) as the quaternary system of solvent strength 1.7. The quaternary system with solvent strength 1.0 is inferior, because it needs a Max k of 9 for a Min Res of 1.5 and cannot give much higher maximum values of Min Res than the water–ACN–THF and the water–ACN–THF and the water–THF–methanol ternary system, which produce the same Min Res values in shorter analysis times (Table 3).

For the full quaternary system no response surfaces can be shown, which in our opinion is a disadvantage. The quaternary system encompasses, of course, all the possibilities of the previously discussed systems, which are factor subspaces of the quaternary system. The performance of the quaternary system with respect to both separation and analysis time is best displayed by the MCDM diagram (not shown) and the supplementary list of eluent compositions, of which a few are given in Table 4. The MCDM diagram of the full quaternary system is nearly identical with the best points in Fig. 12. It is remarkable that many PO points have ternary eluent compositions. The fastest separations up to a Min Res value of 2.2 are again given by the ternary system water–methanol–ACN and the higher values of the Min Res (Table 4) are realized by either the water–ACN–THF (Fig. 12, backslashes) or the water–methanol–THF system (Fig. 12, dashes) in almost the same analysis times as needed by the full quaternary system.

TABLE 4

ELUENT COMPOSITIONS OF A FEW SELECTED PO POINTS IN THE MCDM DIAGRAM OF THE QUATERNARY MOBILE PHASE SYSTEM SHOWN IN FIG. 13

Water	*Methanol*	*ACN*	*THF*	*Max k*	*Min Res*
0.38	0.44	0.18	0.00	1.534	0.283
0.40	0.34	0.26	0.00	1.593	0.492
0.42	0.24	0.34	0.00	1.719	0.793
0.44	0.16	0.40	0.00	1.919	1.137
0.46	0.10	0.44	0.00	2.171	1.481
0.50	0.04	0.46	0.00	2.764	1.822
0.56	0.00	0.44	0.00	3.990	2.202
0.56	0.08	0.06	0.30	4.000	2.242
0.58	0.08	0.00	0.34	4.830	2.562
0.60	0.02	0.04	0.34	5.171	2.578
0.60	0.02	0.00	0.38	5.238	2.643
0.60	0.04	0.02	0.34	5.359	2.671
0.60	0.06	0.00	0.34	5.569	2.763
0.62	0.00	0.06	0.32	5.938	2.774
0.62	0.06	0.02	0.30	6.576	3.010
0.64	0.00	0.00	0.36	7.241	3.186
0.66	0.00	0.10	0.24	8.347	3.424
0.66	0.02	0.10	0.22	8.611	3.511
0.68	0.00	0.08	0.24	10.077	3.835
0.68	0.00	0.06	0.26	10.165	3.853
0.70	0.00	0.10	0.20	12.017	3.963

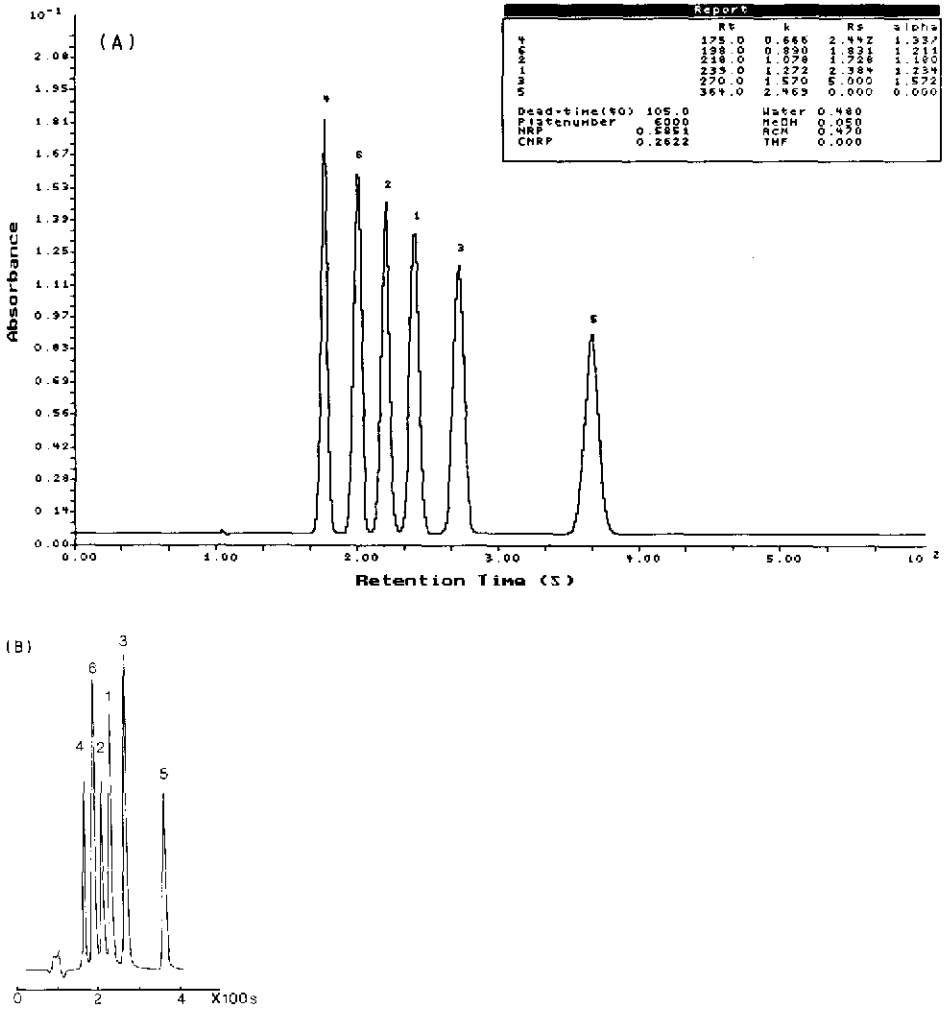

Fig. 13. (A) Similated chromatogram. Gaussian peaks of constant area assumed; plate number 6000; dead time 105 s; mobile phase composition is water–methanol–ACN (0.48:0.05:0.47). See Table 1 for peak identities; see Table 5 for predicted capacity factors, Min Res and Min Alpha. (B) Experimental chromatogram for the same mobile phase composition as in A. See Table 1 for peak identities; see Table 5 for measured capacity factors.

The overall comparison of the results obtainable by the three non-isoeluotropic eluent systems with the results of the full quaternary systems suggests that for relatively simple separations, as in the present example, non-isoeluotropic ternary systems together match the performance of the quaternary system. The individual non-isoeluotropic ternary system matches partly the performance of the quaternary system within a limited part of the Max k range (Table 3).

As experimental verification, a predicted and an experimental chromatogram are depicted in Fig. 13. The predicted and the experimental values of the capacity

TABLE 5

COMPARISON OF EXPERIMENTAL AND PREDICTED RESULTS FOR THE CAPACITY FACTOR, MIN ALPHA AND MIN RES VALUES

Solute[a]	*Capacity factor*		*Min Alpha*		*Min Res*	
	Exptl.	*Pred.*	*Exptl.*	*Pred.*	*Exptl.*	*Pred.*
PE (4)	0.714	0.666	1.24	1.34	1.85	2.44
pC (6)	0.886	0.890	1.21	1.21	1.79	1.83
BA (2)	1.069	1.078	1.23	1.18	2.21	1.73
BN (1)	1.320	1.272	1.21	1.23	2.16	2.38
NB (3)	1.594	1.570	1.58	1.57	5.85	5.76
TL (5)	2.520	2.469	–	–	–	–

[a] For abbreviations, see Table 1.

factors, of the Min Res and of the Min Alpha are compared in Table 5. The predicted and the experimental values of the capacity factors differ by not more than 4.0%, except for the solute with the smallest capacity factor, where the difference is 7.2%. Also, the agreement between the predicted and experimental values of Min Alpha is good, but the difference between the experimental and predicted values of the Min Res is more than 10% in three instances. For the small capacity factor the difference may be explained by a propagation of error analysis, which shows the great influence of inaccuracies in the measurement of the dead time on the determination of small values of the capacity factor[30]. Moreover, a preliminary analysis of the propagation of error and calculations on similated chromatograms showed that the relative error in the resolution can be as high as 35% for standard deviations of 0.05 units in the capacity factor and a difference of 0.2 units between the capacity factors for which the resolution is calculated. This aspect of the Min Res needs further investigation, but indicates that the precise prediction of the resolution may be difficult owing to the propagation of errors.

5. CONCLUSIONS

A quadratic model of the capacity factor can predict well the capacity factors of simple solutes in isocluotropic and non-isocluotropic ternary and quaternary systems consisting of water and organic modifiers.

As an optimization method, the optimization of three isoeluotropic ternary systems is inferior to that of one isoeluotropic quaternary or one non-isocluotropic ternary mixture, because the latter methods provide more information for the same experiment effort.

The optimization of isoeluotropic quaternary systems requires an *a priori* decision with regard to the solvent strength. If the fraction of water in an isocluotropic quaternary system is changed in order to change the solvent strength, *i.e.*, the ratio of the modifiers is kept constant, not only the solvent strength but also the selectivity may change. As the solvent strength decreases, the maximum analysis time increases and the resolution tends to increase. An incorrect choice for a low value of the solvent strength favours higher resolution but hampers the realization of the minimum analysis time.

The optimization of non-isoeluotropic ternary systems requires an *a priori* decision with regard to the nature of the two modifiers which are to be selected. If the fraction of water in a ternary system is changed, the solvent strength and solvent selectivity can vary considerably. An incorrect choice of the modifiers may hamper the realization of maximum resolution but the realization of minimum analysis times remains possible.

If the results of the optimization of an isoeluotropic quaternary system or of a non-isoeluotropic ternary system are unsatisfactory, both optimization methods can be expanded to construct quadratic models of the logarithm of the capacity factor for the full quaternary mobile phase system. Only four additional experiments are necessary to locate the global maximum with respect to resolution and analysis time and to find the solvent compositions of the PO points in a truncated part of the tetrahedron.

The influence of the propagation of errors on the variability of the resolution needs further investigation.

6. SUMMARY

The optimization of different mobile phase systems is compared using statistical mixture designs. Isoeluotropic and non-isoeluotropic ternary and quaternary mobile phase mixtures have been optimized with regard to selectivity and analysis time. For a sample consisting of six benzene derivatives, the logarithm of the capacity factor of each solute is modelled in a full quaternary mobile phase system consisting of water and three organic modifiers. From the models, different separation criteria such as the resolution or selectivity of the worst separated pair of peaks, *i.e.*, the Min Res or Min Alpha, respectively, are calculated. Also, the capacity factor of the last peak, the Max k, can be predicted as a measure of analysis time. Response surfaces that show the different criteria as function of the different mobile phase systems are plotted and evaluated by multi-criteria decision making (MCDM).

It is concluded that the optimization of isoeluotropic ternary mobile phase systems consisting of mixtures of two pseudo-components of equal solvent strength is inferior to that of isoeluotropic quaternary mobile phase systems consisting of mixtures of three pseudo-components of equal solvent strength or to that of non-isoeluotropic ternary systems consisting of mixtures of water and two organic modifiers. The latter two optimization methods do not guarantee that the global maximum with respect to the Min Res of the full quaternary system consisting of mixtures of water and three organic modifiers will be found.

On the one hand, the isoeluotropic quaternary system does not use variation of the water fraction to influence the selectivity. On the other, a mixture of three modifiers and water may provide a wider range of selectivity than a mixture of two modifiers and water. Both optimization methods predict and realize a good separation of a test sample in a short analysis time provided that the solvent strength of the isoeluotropic quaternary system is properly chosen.

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