

OPTIMIZATION OF MOBILE PHASE COMPOSITION IN LIQUID CHROMATOGRAPHY

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A simple method is suggested for the optimization of the mobile phase composition in isocratic reversed-phase liquid chromatography. The function $f = \sum_i (R_{s,i} - R_s^0)^2$, where $R_{s,i}$ is the attained resolution for two successive peaks and R_s^0 is the desired resolution for the chosen time of analysis (hence, a chosen capacity ratio of the last component eluted), was used as the chromatographic response function. The minimum of this function, corresponding to the optimum state, can be found rapidly by the statistical simplex method. This approach was applied to the separation of Cu, Co, Ni, Pb, and Hg diethyldithiocarbamates in a reversed-phase system with silica gel-C₁₈.

Attempts to employ non-chromatographic data for the prediction of the liquid-chromatographic behaviour of solutes have not met with much success. Therefore, optimization of a chromatographic separation still only means optimization of the proven experimental conditions used¹⁻³.

The well-known formula

$$R_s = (1/4) (\alpha - 1) n^{1/2} k' / (1 + k') \quad (1)$$

shows that the resolution is affected by the selectivity of the chromatographic system, column efficiency, and capacity ratios of the substances to be separated. Since these three parameters affect each other, all of them (and some others) have to be taken into account for optimization⁴. The optimization can be based on empirical, statistical or theoretical methods⁴. In the statistical methods, the simplex approach is usually applied.

In practice, the analyst has available a given column and he or she is required to perform the analysis in a reasonable time. Thus both the column efficiency and the capacity ratio of the longest-retention-time component are given, and only the mobile phase composition (in the isocratic mode), on which the separation factor α depends, remains for optimization. After the optimum mobile phase composition has been established, the column efficiency can be improved by finding the optimum mobile phase flow rate.

Taking into account our previous experience we decided to use the statistical simplex method for the mobile phase composition optimization. This approach has been used for the optimization of liquid chromatographic separation by Watson and Carr⁵ and by other authors^{6,7}. By the simplex algorithm the experimental conditions are varied so that the response followed is iteratively improved. A chromatographic response function (CRF) has been proposed⁸; its general form is

$$f = \sum_i w(P_i, P_o) + z(t_i, t_o), \quad (2)$$

where the subscripts i and o refer to the attained and desired separation of two neighbouring peaks, respectively, or to the attained and desired time of analysis, respectively.

Consider a three-component mobile phase (with components A, B, C), the composition of which is determined by the volume fractions φ_A , φ_B , φ_C . The dependence of the response surface (which need not be planar) on the mobile phase composition can be described by a simplified cubic equation,

$$f = \beta_A\varphi_A + \beta_B\varphi_B + \beta_C\varphi_C + \beta_{AB}\varphi_A\varphi_B + \beta_{AC}\varphi_A\varphi_C + \beta_{BC}\varphi_B\varphi_C + \beta_{ABC}\varphi_A\varphi_B\varphi_C. \quad (3)$$

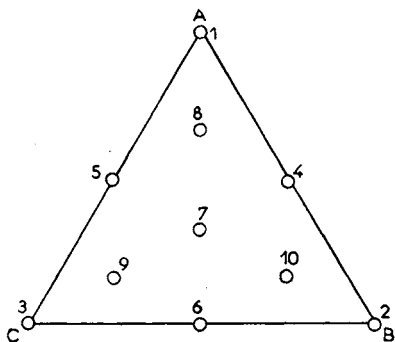


FIG. 1

Simplex method of optimization of properties of a three-component mixture. The points indicate the volume fractions of components A, B, and C

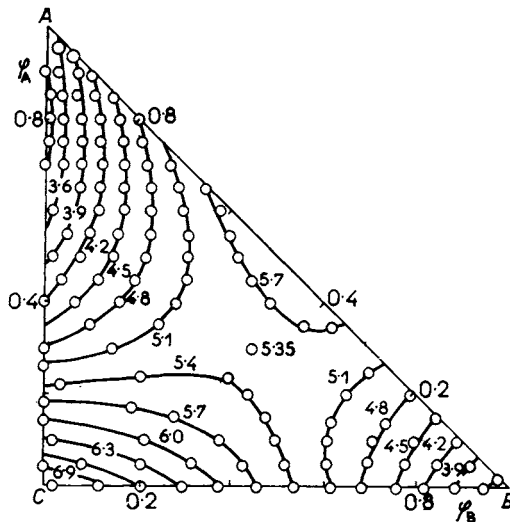


FIG. 2

Dependence of the optimized function f on the $A + B + C$ mixture composition. The composition is indicated by the volume fractions of the components, the figures in triangles give the f values

Snee has suggested a procedure⁹ in which the β coefficients are determined in ten steps (*i.e.*, ten measurements), of which seven serve to determine the seven β 's and in three the fit of the model to the experiment is tested. Fig. 1 shows the compositions of three-component systems for which the response values f are determined. The coefficients in Eq. (3) then are determined as⁹

$$\beta_A = f_A \quad (4)$$

$$\beta_B = f_B \quad (5)$$

$$\beta_C = f_C \quad (6)$$

$$\beta_{AB} = 4f_{AB} - 2(f_A + f_B) \quad (7)$$

$$\beta_{AC} = 4f_{AC} - 2(f_A + f_C) \quad (8)$$

$$\beta_{BC} = 4f_{BC} - 2(f_B + f_C) \quad (9)$$

$$\beta_{ABC} = 27f_{ABC} - 12(f_{AB} + f_{AC} + f_{BC}) + 3(f_A + f_B + f_C) \quad (10)$$

The fit of the model to the reality is evaluated in terms of the difference between the observed responses and those calculated for steps 8–10 according to Fig. 1.

Deviations of the model from the reality are expressed by the variance

$$s_1^2 = (1/r) \sum_{i=1}^r (f_i - f_o)^2, \quad (11)$$

the error of measurement, found by repeated measurements of the response in each step, is expressed by the variance

$$s_2^2 = [1/(N - b)] \sum_{i=1}^b (n_i - 1) s_i^2 \quad (12)$$

(f_i is the observed and f_o , the calculated response in the i -th step; s_i^2 is the variance for n_i times repeated measurement in the i -th step).

The statistical significance of the ratio of the two variances, s_1^2/s_2^2 , is tested by means of the F -criterion (the numbers of degrees of freedom are $v_1 = r - 1$, $v_2 = N - b$).

This optimization approach, known from recent works, was used to establish the optimum mobile phase composition for RP-LC separation of Cu(II), Co(II), Ni(II), Pb(II), and Hg(II) diethyldithiocarbamates on a column of Spheron SI C 18, employing a newly suggested CRF.

EXPERIMENTAL

Chemicals

Methanol was purified by rectification; its spectral transmittance, in a 1 cm cell, was better than 95% at 250 nm and 100% at 260 nm. Dioxane was purified by boiling with an alkaline solution of potassium permanganate and rectification ($T_{250} = 50\%$, $T_{>280} = 90\%$ at $l = 1$ cm). Acetonitrile *p.a.* (Avondale Lab., England) and pure (Apolda, GDR) was used without purification ($T_{>250} = 95\%$ at $l = 1$ cm). Tetrahydrofuran was dried over potassium hydroxide and rectified with sodium ($T_{260} = 70\%$, $T_{>280} > 90\%$ at $l = 1$ cm). Solutions of the metal diethyldithiocarbamates were obtained from aqueous solutions of the nitrates or perchlorates at pH 7.6 (citrate buffer) in the presence of sodium diethyldithiocarbamate in excess by extraction into purified chloroform, evaporation in a rotary vacuum evaporator, and dissolution of the residue in a small volume of tetrahydrofuran.

The mobile phases were prepared by mixing the freshly purified solvents, *viz.* methanol (A), acetonitrile (B), and tetrahydrofuran (C) or dioxane (D). The eluting agents were degassed by 5 min boiling under reflux and rapid cooling to room temperature.

Apparatus

A Varian Model 4000 liquid chromatograph (Varian, Palo Alto, USA) was used. The mobile phase was pumped under nitrogen pressure of max. 7 MPa and samples were injected by means of an 701 N Hamilton microsyringe through a septum injector with silicone rubber septa (Laboratorní přístroje, Prague). A photometric detector of Varian ($\lambda = 254$ and 280 nm, cell volume 8 μ l) was used. The column (250 \times 4 mm) was packed with Separon SI C 18, spherical silica gel with chemically bonded C₁₈ groups, $d_p = 10$ μ m, specific surface area 150–300 m² g⁻¹ (Laboratorní přístroje, Prague).

RESULTS AND DISCUSSION

It was found, based on the parameters of the instrument used, that for the time of analysis to be held reasonably short, the chelate capacity ratio should not exceed the value of $k = 7$.

In order to be able to study the differences in the selectivity of the interactions in which the mobile phase is involved and, on their basis, to establish the optimum composition of the mobile phase, we had to keep the eluting strength constant for all the solutions used; this was achieved by using binary mixtures of organic solvents with water in the appropriate proportions.

A mixture of methanol with water, with $\varphi(\text{methanol}) = 0.82$, was used for the mercury chelate, exhibiting the longest retention time ($k \approx 7$); the compositions of the binary mixtures of water with acetonitrile, tetrahydrofuran, and dioxane, respectively, possessing the same eluting strength as the above methanol–water mixture were calculated^{10,11} from the solubility parameters δ of the solvents by means of the relation

$$\delta_{\text{mix}} = \delta_A \varphi_A + \delta_{\text{H}_2\text{O}} \varphi_{\text{H}_2\text{O}} \quad (13)$$

The compositions of the binary mixtures satisfying the condition that the capacity ratio of the last-eluting component is $k \approx 7$ were also sought experimentally (Table I); these values agree well with those calculated by Eq. (13) in all cases except for the tetrahydrofuran-water system. The experimental φ_{org} values were found from the $\log k$ vs φ_{org} plots.

In addition to the solubility parameter, other quantities describing the solvent strength in liquid chromatography, such as Rohrschneider's polarity P' (refs^{12,13}) or the RP-LC solvent strength S (ref.¹⁴), can in principle also be employed for the calculation of the compositions of the isoelectrotonic aqueous binary mixtures; in our case of the metal diethyldithiocarbamates, however, the compositions calculated by means of these quantities were in a poorer agreement with the experiment than those derived by using the solubility parameter.

The aim of the optimization procedure was to find mixtures of methanol, acetonitrile, tetrahydrofuran, and water (A + B + C) and methanol, acetonitrile, dioxane, and water (A + B + D) with which the diethyldithiocarbamates of five metals, *viz.* Cu, Co, Ni, Pb, and Hg, are best separated on a column packed with Separon SI C 18.

The CRF used by us for characterizing the quality of the mixture separation was defined¹⁵ as

$$f = \sum_i (R_{s,i} - R_s^0)^2, \quad (14)$$

where $R_{s,i}$ is the attained resolution of two successive peaks and R_s^0 is the desired resolution. Since the chromatographic output data were not subjected to computer processing (*e.g.*, peak deconvolution), the value of 1.5 was taken for R_s^0 . Function f acquires the optimum value of $f = 0$ if all the four pairs of neighbouring peaks are perfectly resolved, whereas if all peaks of the five compounds overlap, the function has the value of $f = 9$.

TABLE I

Compositions of binary mixtures of organic solvents with water exhibiting identical eluting strength

Organic solvent (label)	φ_{org} in mixture	
	calculated ^a	found
Methanol (A)	0.82	0.82
Acetonitrile (B)	0.72	0.73
Tetrahydrofuran (C)	0.56	0.46
Dioxane (D)	0.59	0.61

^a Calculated by means of solubility parameter.

The values of function f obtained from the experimental data are given in Tables II and III for the A + B + C and A + B + D systems, respectively.

The dependence of the response f on the mixture composition can be written as

$$f = 3.43\varphi_A + 3.41\varphi_B + 7.25\varphi_C + 8.68\varphi_A\varphi_B - 6.08\varphi_A\varphi_C + \varphi_B\varphi_C + 4.41\varphi_A\varphi_B\varphi_C \quad (15)$$

TABLE II

Values of the optimized function f for A + B + C mixtures of various composition

Step No	Mobile phase composition			f^a
	φ_A	φ_B	φ_C	
1	1	0	0	3.43
2	0	1	0	3.41
3	0	0	1	7.25
4	0.5	0.5	0	5.59
5	0.5	0	0.5	3.82
6	0	0.5	0.5	5.58
7	0.33	0.33	0.33	5.26
8	0.50	0.25	0.25	5.54
9	0.17	0.66	0.17	4.85
10	0.17	0.17	0.66	5.75

$$^a f = \sum_i (R_{s,i} - R_s^0)^2.$$

TABLE III

Values of the optimized function f for A + B + D mixtures of various composition

Step No	Mobile phase composition			f^a
	φ_A	φ_B	φ_D	
1	1	0	0	3.43
2	0	1	0	3.41
3	0	0	1	7.72
4	0.5	0.5	0	5.59
5	0.5	0	0.5	5.09
6	0	0.5	0.5	7.60
7	0.33	0.33	0.33	5.34
8	0.66	0.17	0.17	4.52
9	0.17	0.66	0.17	5.60
10	0.17	0.17	0.66	6.31

$$^a f = \sum_i (R_{s,i} - R_s^0)^2.$$

for the A + B + C system, and

$$f = 3.43\varphi_A + 3.41\varphi_B + 7.72\varphi_D + 8.68\varphi_A\varphi_B - 1.94\varphi_A\varphi_D + \\ + 8.26\varphi_B\varphi_D - 31.86\varphi_A\varphi_B\varphi_D \quad (16)$$

for the A + B + D system.

For testing the adequacy of these relations for the chromatographic system in question, three f values were calculated for each mixture and compared with the experimental responses (Table IV). For the A + B + C mixture (methanol, acetonitrile, tetrahydrofuran, water), the variances (Eqs (11), (12)) were $s_1^2 = 0.1475$, $s_2^2 = 0.0469$; $F = s_1^2/s_2^2 = 3.145$, $F_{crit} = 3.385$ ($v_1 = 2$, $v_2 = 25$); for the A + B + D system (tetrahydrofuran replaced by dioxane), $s_1^2 = 0.0463$, $s_2^2 = 0.0158$, $F = 2.930$, $F_{crit} = 3.467$ ($v_1 = 2$, $v_2 = 21$). Hence, $F < F_{crit}$ in both cases, which implies that at the $\alpha = 0.05$ significance level the error of the model, characterized by variance s_1^2 , does not exceed statistically significantly the error of measurement, characterized by variance s_2^2 .

The f values were calculated by a computer for various mixtures based on Eqs (15) and (16). The dependences of the CRF (*i.e.*, function f) on the mixture compositions are shown in Figs 2 and 3, from which the optimum compositions of the eluting agents, with the lowest f values and thus leading to the best resolution of the solute peaks, can be determined. For the A + B + C system, the best separation was achieved with a mixture with $\varphi_A = 0.80$, $\varphi_C = 0.20$, hence, containing 65.6% (v/v)

TABLE IV

Comparison of calculated and experimental f values for A + B + C and A + B + D mixtures

Mobile phase composition			f_{calc}^a	f_{found}
φ_A	φ_B	$\varphi_{C(D)}$		
A + B + C mixtures				
0.50	0.25	0.25	4.91	5.54
0.17	0.66	0.17	5.06	4.85
0.17	0.17	0.66	5.71	5.75
A + B + D mixtures				
0.66	0.17	0.17	4.54	4.52
0.17	0.66	0.17	5.38	5.60
0.17	0.17	0.66	6.61	6.31

$$^a f = \sum_1 (R_{s,i} - R_s^0)^2$$

methanol, 9.2% (v/v) tetrahydrofuran, and 25.2% (v/v) water; the corresponding f value was 3.20. The chromatogram obtained using this mixture is shown in Fig. 4. In spite of the fact that the mobile phase composition was at its optimum, the five chelates were not separated perfectly because of the low efficiency of the column used ($n = 2\,200$ for solute with $k = 1.36$).

For the A + B + D system, the best resolution, characterized by $f = 3.41$ (Fig. 3), is achieved by using a solution of B alone, *i.e.* an acetonitrile–water binary system with 73% (v/v) acetonitrile.

The use of the CRF in the form suggested, $\sum_i (R_{s,i} - R_s^0)^2$, is equivalent to the requirement that in the optimum, the deviations of the observed resolution from the desired resolution, both positive and negative, should be minimum for all the solute pairs. The weight coefficients shown in Eq. (2) were not considered, the separation significance being identical for all chelate pairs. The time factor (the second right-hand

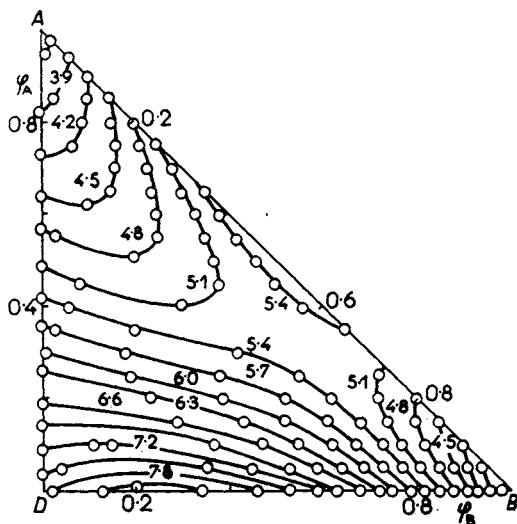


FIG. 3

Dependence of the optimized function f on the A + B + D mixture composition. The composition is indicated by the volume fractions of the components, the figures in triangles give the f values

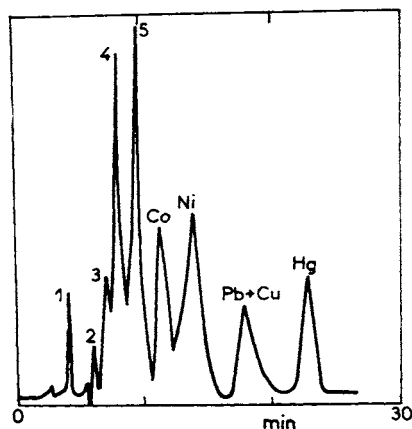


FIG. 4

Separation of Cu, Co, Ni, Pb, and Hg diethyldithiocarbamates using the mobile phase of optimum composition, *viz.* φ (methanol) = 0.656, φ (tetrahydrofuran) = 0.0992, φ (H₂O) = 0.252. Column 250 × 4 mm packed with Separon SIC 18, $d_p = 10\ \mu\text{m}$; $F_m = 0.48\ \text{ml min}^{-1}$, $t_M = 2.81\ \text{min}$; injected: 5 μl of chelate solution in tetrahydrofuran. Peaks: 1, 2 impurities in tetrahydrofuran, 3–5 reagent and its decomposition products

term in Eq. (2)) had not to be included in the CRF because the time of analysis was sufficiently limited by the requirement that the capacity ratio of the longest-retention-time chelate be $k \approx 7$.

The optimization method suggested is experimentally simple because data from ten chromatograms are sufficient for the evaluation, and the minimum on the response surface can be easily found by means of a computer with an alphanumeric or graphic output.

LIST OF SYMBOLS

- b total number of steps
 f chromatographic response function (CRF)
 F Snedecor's criterion ($F = s_1^2/s_2^2$)
 k capacity ratio
 k' average of capacity ratios of two neighbouring peaks, $k' = (k_1 + k_2)/2$
 n number of theoretical plates of column
 n_i number of repeated measurements in the i -th step of the simplex
 N total number of experiments in all steps
 r number of steps in the simplex algorithm
 s standard deviation (estimate)
 w statistical weight
 z statistical weight
 α separation factor, $\alpha = k_2/k_1$
 β coefficients in cubic equation (3)
 δ solubility parameter
 φ_A volume fraction of component A

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