MOBILE PHASE OPTIMIZATION IN THIN LAYER CHROMATOGRAPHY (TLC)

Claudia Cimpoiu and T. Hodisan[1]

"Babes-Bolyai" University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos, 3400 Cluj-Napoca, Romania

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SUMMARY

This review presents the strategies for mobile phase optimization that either have been developed for or have been applied to planar chromatography. In thin layer chromatography some form of optimization is generally necessary if complete separation of all components in a sample is required and if the number of these is larger than a small fraction of the spot capacity of the system. The selection of the solvent system is one of the most important components of an optimization strategy and indeed is often the only component seriously considered.

[1] Corresponding author

1. INTRODUCTION

Mobile phase optimization is highly important in thin layer chromatography (TLC). From the early days of chromatography as an analytical tool for the separation and determination of multi-component mixtures, great effort has been made in the field of optimization because of specific problems encountered in the liquid chromatography. With the widespread availability of computers in analytical laboratories, the topic became more and more preferred /1-5/ and today several reviews can be consulted both for liquid chromatography (LC) as well as for TLC /4, 5/ and a very few for more general cases /6/.

Unlike other chromatographic methods, where there are many factors or variables that may be manipulated to improve system performance, TLC methods are relatively restricted methods. Although the system is complex, many of the factors are fixed by practical or theoretical considerations and the analyst may only manipulate solvent composition, spot size, temperature and in certain cases, relative humidity /7/.

Relative humidity is an experimental variable that is somewhat difficult to vary within narrow ranges and it is therefore not practical to choose it as an optimization factor /7/.

There is no reason to use temperature as an optimization variable in isothermal conditions, since the minimum analysis time would be practically insensitive to temperature changes in the normal - temperature - work range for a given resolution /8/.

Since many criteria used to judge chromatographic performances are based on resolution, it is better to use the spot size value that gives the better resolution, and therefore to vary other factors when optimizing the system.

The most important factor that must be considered in the optimization of a thin layer chromatography system is the composition of the mobile phase.

The procedure to optimize an analytical method is /10/:

I. the selection of experimental variables which must be optimized and how the optimization will be measured;
II. the determination of the initial experimental conditions and how they will be changed in subsequent experiments;
III. the evaluation of optimization results.

2. SOLVENT CLASSIFICATION

The choice of the mobile phase to realize a given separation constitutes a very important stage in thin layer chromatography.

A good selection of the mobile phase system will allow the separation of any mixture, and then the choice of an elution system may be considered the most important component of an optimization strategy and indeed is often the only component seriously considered.

The solvents used as the mobile phase must fulfill the following conditions /11/: good polarity for the mixture's separation, low velocity, high purity, stability, compatibility with the detection system.

Several criteria are involved in the classification of solvents used in chromatography. A series of authors made different classifications of solvents on the basis of some criteria such as: solvent strength parameter, polarity index, Hildebrand's solubility parameters.

2.1. Classification of Solvents Using the Solvent Strength Parameter, ε^0. Eluotropic Series.

The solvent strength refers to the ability of a solvent or solvent system to elute a solute. ε^0 has been defined by Snyder /12/ as "the adsorption energy of the solvent per unit area of a standard activity surface". Using this parameter the solvents have been classified on the eluotropic series for various polar adsorbents: alumina, silica gel, Florisil, magnesia, etc.

Solvent selectivity may be defined as the ability of a particular solvent system to separate a pair of compounds that are not separated in other systems.

The strength and selectivity of a solvent are often treated as independent variables. First, the optimum strength is found, usually by diluting a solvent with a weak solvent and then the optimum separation is effected by substituting other solvents or solvent systems of the same strength but of different selectivity. It should however be noted that strength and selectivity are often related.

Snyder /12/ has shown that there is a correlation between the ε^0 values for a certain polar adsorbent and alumina:

$$\varepsilon^0_{SiO_2} = 0.77\varepsilon^0_{Al_2O_3}; \quad \varepsilon^0_{Florisil} = 0.52\varepsilon^0_{Al_2O_3}; \quad \varepsilon^0_{MgO} = 0.58\varepsilon^0_{Al_2O_3} \qquad (1)$$

The relationship between the partition coefficient, α, and the eluent composition in the adsorption chromatography is /13/:

$$\alpha = \alpha_p \cdot 10^{-E\varepsilon^0 A_i} \tag{2}$$

where α_p is the value of the partition coefficient when pentane is used as eluent; E, the superficial energy of adsorbent and A_i, the area of component adsorption on the surface.

There are equations that express the strength of a binary eluent, $\varepsilon_{1,2}$ /14/ and the strength of a ternary eluent, $\varepsilon_{1,3}$ /15/:

$$\varepsilon^0_{1,2} = \varepsilon_1 + \frac{\log\left[x_2 \cdot 10^{\beta n_2(\varepsilon_2-\varepsilon_1)} + 1 - x_2\right]}{\beta n_2} \tag{3}$$

$$\varepsilon^0_{1-3} = \varepsilon_2 + \frac{\log\left[x_3 \cdot 10^{\beta n_3(\varepsilon_3-\varepsilon_2)} + x_2\right]}{\beta n_3} \tag{4}$$

where n_2, n_3 are the effective molecular area of an adsorbent solvent molecule S_2 and S_3 respectively, and β represents the adsorbent's surface activity function.

These equations predict that the addition of even a small quantity of a stronger solvent to a weak solvent will have a substantial effect on the strength of the mixture while the effect of incremental additions at higher concentrations of the stronger solvent is less pronounced.

On the basis of these considerations an infinite number of eluotropic series can be established. By substituting one of the solvents with a solvent that has similar n and ε^0 parameters, we will obtain a new series of not very different eluotropic properties, but with another selectivity. This reasoning is based on the fact that by changing the eluent compositions we modify the component - solvent interactions.

The composition of binary solvent systems that have the same strength (equieluotropic systems) can be obtained by means of nomograms /16/.

2.2. Classification of Solvents Using the Polarity Index, P'

Rohrschneider /17/ proposed a solvent classification on the basis of the solubility parameter of six substances in eighty-two common solvents. But

the Rohrschneider coefficients did not make it possible to distinguish between the solvent strength and the solvent selectivity.

Snyder /18, 19/ has derived the P′ polarity index based on the individual gas - liquid distribution coefficient of ethanol, dioxane and nitromethane in a large number of solvents as determined by Rohrschneider /20/. P′ reflects the solvent capacity to interaction with different solutes,

$$P' = \log\left(K_g''\right)_e + \log\left(K_g''\right)_d + \log\left(K_g''\right)_n \qquad (5)$$

where subscripts e, d and n refer to ethanol, dioxane and nitromethane and K_g'' is a constant that is proportional to the energy of interaction of the solute with a given solvent. Correction is made for the molar volume of both the solute and the solvents.

The selectivity parameters x_e, x_d and x_n are defined by dividing the corresponding log K_g'' values by P′ and they reflect the "relative ability of a solvent to function respectively as a proton acceptor, a proton donor, or a strong dipole interactor" /18/.

Solvents can be divided into eight groups by plotting the values of x_e, x_d and x_n on triangular axes and it is assumed that, within each group, solvents are equivalent in selectivity. The values of x_e, x_d and x_n for some solvents are presented in Table 1.

Snyder /19/ has shown that "while there are a large number of solvents that can be used for this purpose, the use of three properly chosen polar solvents plus some nonpolar diluent should provide almost all of the selectivity available from the complete list of solvents of known selectivity". Snyder suggested that the short list would include ethyl ether (group I), methylene chloride (group V) and chloroform (group VIII) with either hexane or carbon tetrachloride as a nonpolar diluent. Other authors suggested other solvents from the selectivity triangle /21-25/.

The parameter P′ should strictly be used only for nonaqueous systems and for a mixture of solvents A and B it can be calculated with the following equation:

$$P = \Phi_A P_A + \Phi_B P_B \qquad (6)$$

where Φ_A and Φ_B are the volume fractions of solvents A and B ($\Phi_A + \Phi_B = 1$), P_A and P_B are the polarity index of pure solvents A and B. The parameter S, derived by an empirical method /26/, should be used with aqueous systems.

Table 1. Classification and characterization of the solvents.

	Solvent Group	Individual Strength S_i	x_e	x_d	x_n
-	n-Hexane	0,1	-	-	-
I	di-n-Butyl ether	2,1	0,44	0,18	0,38
	Diisopropyl ether	2,4	0,48	0,14	0,38
	Methyl-t-butyl ether	2,7	0,49	0,14	0,36
	Diethyl ether	2,8	0,53	0,13	0,34
II	n-Butanol	3,9	0,59	0,19	0,25
	i-Propanol	3,9	0,55	0,19	0,27
	n-Propanol	4,0	0,54	0,19	0,27
	Ethanol	4,3	0,52	0,19	0,29
	Methanol	5,1	0,48	0,22	0,31
III	Tetrahydrofuran	4,0	0,38	0,20	0,42
	Pyridine	5,3	0,41	0,22	0,36
	Methoxyethanol	5,5	0,38	0,24	0,38
	Dimethylformamide	6,4	0,39	0,21	0,40
IV	Acetic acid	6,0	0,39	0,31	0,30
	Formamide	9,6	0,36	0,23	0,30
V	Dichlormethane	3,1	0,29	0,18	0,53
	Ethylene chloride	3,5	0,30	0,21	0,49
VI	Ethyl acetate	4,4	0,34	0,23	0,43
	Methyl-ethyl ketone	4,7	0,35	0,22	0,43
	Dioxane	4,8	0,36	0,24	0,40
	Acetone	5,1	0,35	0,23	0,42
	Acetonitrile	5,8	0,31	0,27	0,42
VII	Toluene	2,4	0,25	0,28	0,47
	Benzene	2,7	0,23	0,32	0,45
	Nitrobenzene	4,4	0,26	0,30	0,44
VIII	Chloroform	4,1	0,25	0,41	0,33
	Nitromethane	6,0	0,28	0,31	0,40
	Water	10,2	0,37	0,37	0,25

Every modern text on chromatography has included this eight group classification scheme, without indicating that the accuracy of the classification has not been tested.

2.3. Classification of Solvents Using Hildebrand's Solubility Parameters

The Hildebrand solubility parameter defines a quantitative scale of solvent polarities:

$$\delta_i = \left(\frac{\Delta E_i^v}{V_i} \right)^{1/2} \tag{7}$$

where E^V is the vaporization energy per mole of pure liquid i and V_i is the molar volume. The solubility parameter, δ_1, is useful only for nonpolar systems, but it has been extended to polar substances /27/. ΔE_i^V represents the energy per mole required to overcome interactions between i molecules in the pure liquid state during the process of evaporation. This energy can be expressed by a sum of four types of interaction energies per mole:

$$\Delta E^v = \delta^2 V = \left(E_{ii}\right)_d^v + \left(E_{ii}\right)_{in}^v + \left(E_{ii}\right)_o^v + \left(E_{ii}\right)_{hb}^v \tag{8}$$

where $(E_{ii})d^v$ represents dispersion; $(E_{ii})_{in}^v$, induction; $(E_{ii})_o^v$, dipole orientation, and $(E_{ii})_{hb}^v$, hydrogen bonding.

Each of these energy terms can be expressed as a product of specific solubility parameters /28/:

$$\delta^2 = \delta_d^2 + 2\delta_{in}\delta_c + \delta_o^2 + 2\delta_a\delta_b \tag{9}$$

and δ_d is a measure of the ability of a substance to participate in dispersive interaction; δ_o, the ability of a species to participate in dipole orientation interactions; δ_{in}, the ability of that species to induce a dipole moment in surrounding molecules; δ_a and δ_b, the ability of that species to function as a proton donor or acceptor, respectively.

A classification of the solvents and adsorbents that can be used to estimate the selectivity in chromatography can be made on the basis of these parameters /7/.

While the solvent strength is given by the global parameter δ, its selectivity is controlled by the specific solubility parameters.

Although there are many solvents having different solubility parameters, the use of a mixture of solvents (binary, ternary or quaternary systems) is very often necessary.

3. MOBILE PHASE SELECTION IN TLC

To choose the mobile phase system we must know the sample composition or at least its nature. The mechanism of separation and then the mobile phase composition is chosen in accordance with the sample nature. So, for nonpolar samples an adsorption separation technique is recommended and for the polar samples the reversed phase partition chromatography most suitable. Samples consisting of ionizable substances suggest the use of ion exchange or partition and the samples that have a high viscosity require the use of steric exclusion chromatography. After having decided on a separation mechanism, the next stage is selection of the stationary phase.

Selection of the separation conditions can be done using the Stahl triangle /3/. This system is quite rigid and does not allow for a real selection of the adsorbent activity.

A more adequate representation is realized by a nomogram that shows a correlation between the polarity of the mixture to be separated, the activity degree of the adsorbent, and the eluent strength /29/.

3.1. Selecting a Mobile Phase in Adsorption TLC

In TLC, the solute retention is dependent on the probability of interaction of the solute with the polar molecules contained in the mobile phase, which in turn is dependent on the concentration of the polar solvent in the mobile phase. The coefficient of distribution of a solute between the two phases in a chromatographic system is defined by means of the ratio between the total forces acting on the solute in the stationary phase and the total forces acting on the solute in the mobile phase. To express the distribution coefficient K in adsorption chromatography, Scott /30/ took into consideration only polar and dispersive interactions, at constant temperature:

$$K = \frac{\left(\Phi_p F_p P_p + \Phi_d F_d P_d\right)_s}{\left(\Phi_p F_p P_p + \Phi_d F_d P_d\right)_m} \qquad (10)$$

where ϕ is a constant, F, the magnitude of the force between the solute molecule and the phase molecule, P, the probability of molecular interaction, and the subscripts s and m designate the stationary and mobile phases, respectively.

For substances insoluble in water it is clear, after long experience with TLC, that only a few solvent mixtures are sufficient for the separation of a considerable number of components on silica gel /31/.

In the separation of drugs good results are obtained with some mixtures of solvents /32/: chloroform / methanol / acetic acid 5% (85 : 10 : 5 v/v), chloroform / concentrated methanol / NH_3 (70 : 25 : 5 v/v) for acid compounds; chloroform ! benzene (1 : 1 v/v) saturated with NH3 and chloroform / methanol / glacial acetic acid (85 : 5 : 10 v/v) for basic compounds; and chloroform / methanol (90 : 10 v/v) for neutral substances.

3.2. Selecting a Mobile Phase in Liquid - Liquid (Partition) TLC

A good choice of mobile and stationary phases in liquid-liquid partition chromatography must entail a capacity factor k value within the range $1 \leq k \leq 10$, which corresponds to a relative migration speed of the component, R_f, in the range $0,50 \geq R_f \geq 0,09$. Polar and nonpolar solvents are used with normal phases, and polar solvents with reversed phases. It is necessary to increase the polarity of the mobile phases when the polarity when the sample polarity increases in order to maintain eluent.

The water in the eluent has an important role in separations on silica gel because it binds to the active sites of the silica gel and reduces their binding strength for solute molecules /33/. A large quantity of water can form a multilayer of water on silica gel /34/, and the stationary phase becomes inadequate for the separation of water - insoluble organic compounds.

To improve the separation of basic compounds we can add small quantities of various salts in the mobile phase such as: NaCl, KCl, $MgCl_2$, etc. The presence of salts in the mobile phase acts to conceal the residual OH groups accessible from the surface of silica gel.

3.3. Selecting a Mobile Phase in Ion - Exchange TLC

Ion exchange involves the substitution of one ion by another through the breaking of some ionic bonds and the forming of new ones, and belongs to the category of double exchange reactions.

Water is the ideal solvent, due to its good solvating and ionizing properties. Sometimes, alcohol is added to increase the selectivity of the separations and the solubility of certain compounds.

Generally, the eluent is a buffer solution and its strength can be modified by addition of an appropriate electrolyte, and its pH is chosen so as to be close to the pK_a value of the components to be separated /35/.

4. MOBILE PHASE OPTIMIZATION

The choice of mobile phase system may be considered the most important component of an optimization strategy and indeed is often the only component seriously considered. In the literature the principles for the choice of the mobile phase system for different classes of substances are described, but there are also cases when systems selected, even if they are correctly used, do not give the expected results. In this case the research worker may resort to "the art of separation" /36/ or to the methods of optimization.

In the literature both simple and more sophisticated methods for the mobile phase optimization are described and some of these methods were realized with the help of computers.

4.1. Simple Methods

An intuitive, trial and error approach to solvent selection is often acceptable when mixtures containing only a small number of components are to be separated.

A great number of solvents can be chosen using different developing chambers such as: Camag Vario KS chamber or multiple development chamber /37/, which allows the simultaneous evaluation of more mobile phase systems.

Another method for the rapid choice of solvent mixtures uses circular planar chromatography /38/. Three different solvent systems are simultaneously fed to the planar chromatographic layer at three inlets uniformly spaced around the centre of the plate.

In the method due to Saunders /39/ the ε^0 values are computed for several binary solvent systems and each is represented by a straight line with a volume fraction scale, which allows reading solvent strength at any

composition against an ε^0 scale. There it is supposed that the selectivity and the solvent strength are independent.

The optimization of ternary and quaternary mobile phase systems can be realized using a method that is based on more simultaneous experiments that used multicomponent isocratic mobile phases /40, 41/.

4.2. Sophisticated Methods

Solvent selection based on experience and chromatographic intuition is suitable for the separation of very simple mixtures but can be very time-consuming when applied to complex mixtures. For the complex mixtures more systematic strategies were elaborated which intend to find the maximum or minimum of a function called "objective function". The chromatographic response function (CRF) reflects the quality of separation in a single number.

4.2.1. Chromatographic Response Function

While no one CRF will ever be entirely satisfactory in all cases and for all chromatographers a great number of CRFs has been designed and tested. A list of them is presented in Table 2 without the pretension to be exhaustive.

In Table 2, R_s is the resolution of every pair of the n-1 adjacent peaks, n-the number of peaks/investigated compounds, m-number of expected peaks, O_i- statistical frequencies observed within a "group", E_i-the theoretical frequency for an ideal distribution, I-amount of information, p_k-the probability of finding a peak in a group, Si-peak overlap, L_{chr} - length of the chromatogram, σ_s, and σ_f the standard deviation of the first and last peaks, $\Delta hR_{f,l}$ - measured intervals between two adjacent peaks, $\Delta hR_{f,t}$ - theoretical intervals between any two adjacent peaks in the case of an ideal separation.

In practice we can say that a given chromatogram is "the optimum" if it fulfills the following conditions /42/: the number of separated components must be maximum; the peak width must be as low as possible; the separation co-ordinates of all individual peaks must be distributed throughout the chromatogram as uniformly as possible; the separation of all adjacent pairs of peaks must be the best, on average, even if the separation is not perfect; the solvent system and the stationary phase used must have a maximum separation potential and the separation time must be the shortest.

To satisfy all these conditions, the preferred CRF is a combined function as weighted sum of simple functions.

Table 2.

Some chromatographic response functions used in mobile phase optimization.

No	Name of Function	Equation	Author	Ref
1	Chromatographic Response Function	$CRF = \sum \ln(n_i)$ (FCR)	Morgan	44, 53
2	Trennungszahl (sep.no.)	$TZ = 2L_{chr}/(\sigma_s + \sigma_f) - 1$	Kaiser	45
3	Amount of Information	$I = -\sum(n_k/n)\lg_2(n_k/n)$	Souto	46
4	χ^2 Function	$\chi^2 = \sum(O_i - E_i)^2/E_i$	Massart	47
5	Discrimination Power	$DP = 1-(2m/n(n-1))$	Massart	48
6	Performance Index	$I_p = \sqrt{\frac{\sum_{i}(\Delta hR_{f,i} - \Delta hR_{f,i})^2}{n(n+1)}}$	Gocan	49
7	Information Power	$I \cdot \bar{R}_s = I \cdot \sum R_i/n$	Naşcu	50
8	Informational Energy	$IE = \sum p_k^2$	Sarbu	51
9	Information Power	$P_{inf} = \sum^2 \log S_i$	Smits	52
10	Separation Number	$SN = \sum \log_2 S_i$	Spencer	54
11	Resolution Product	$PRS = \prod R_i$	Schoen-makers	55
12	Separation Degree	$Y = n/m$	Doudi	56
13	Relative Resolution Product	$RRP = \frac{\prod R_i}{\sum R_i/(n-1)}$	Warren	57

In the previous papers /42, 43/ combined CRFs were used, such as

$$F = a\frac{I_p}{10} + b\frac{10}{I} + c\frac{10}{I\bar{R}_s} + d\frac{1}{100RRP} \quad (11)$$

$$F = a \cdot n + b \cdot I\bar{R}_s + c \cdot 10 / IE + d / \left(I_p + \varepsilon\right) \quad (12)$$

where: a, b, c and d are arbitrary weighting factors; n is the number of components observed as peaks (zones); I, the amount of information (position no. 3 in Table No. 2); R_s, the mean resolution of all adjacent peaks (see equation under no. 7 in Table No. 2); IE, the informational energy (see no. 9 in Table No. 2), I_p the performance index (see no. 6 in Table No. 2); RRP, the relative resolution product (see no. 14 in Table No. 2) and ε, a very small, arbitrary value (10^{-5}).

The number of components entirely separated, n, is a useful quality /58, 59/, but if the separation is not "uniform" i.e. the R_f values of the peaks are not equally distributed, the plate must be longer and the separation time consequently longer. Therefore, this number alone is not entirely satisfactory.

The overall separation of all pairs of adjacent peaks was estimated by using the mean resolution of all consecutive peaks /55/ or by calculating a product of this function with the amount of information /50/. The amount of information, I /46/ and the informational energy, IE /51/ illustrate the multi-component separation using discontinuities of probabilities related to some arbitrary "groups" of retention parameter values. They are not affected by peak widths and therefore not very sensitive, especially for a small number of peaks.

The performance index, I_p, reflects the uniformity of the separation and this function is very useful.

4.2.2. Window Diagrams

This method is due to Laub and Purnell /60/ which used window diagrams in optimization of separation by gas - liquid chromatography. This method has then been widely used, both in gas chromatography and in HPLC, but has hardly been used in planar chromatography /61, 62/. ΔR_f was used as the separation parameter, $\Delta R_f = (k_2 - k_1)/(1 + k_1)(1 + k_2)$, that was plotted against solvent composition.

If all solute pairs are considered, the plot represents a window diagram that identifies the optimum solvent composition. When all solute pairs in a mixture are considered, it is found that the values of $(\Delta R_f)_{max}$, the maximum values of ΔR_f tend to cluster around a particular mole fraction of the binary solvent system. This composition is referred to as the cluster centre of the binary system.

The advantage of this method is that the global optimum can be very easy localised either by eyes or by computers.

A system consisting of three or more solvents allows a large variety of intermolecular interactions and, when optimized, would be expected to yield a better separation than that attainable in a binary mixture of solvents.

The approaches that have been used for optimising such systems are the simplex algorithm, the prisma approach, the overlapping resolution mapping scheme, etc. These are discussed below.

4.2.3. The Sequential Simplex Method

In 1962, Spendley *et al.* /63/ introduced the sequential simplex method and it was then used in analytical chemistry by Long /64/. This method is simple and fast and it can be used in automatic optimization.

The principle of the Simplex algorithm consists in the utilization, in the variable space of objective function, of a geometric figure whose number of vertices is bigger by one than the number of variables. So, a Simplex model in two dimensions is a triangle and in three dimensions is a tetrahedron. The objective function is evaluated in each vertex of the figure and then the direction is searched that is experienced as the most unfavourable vertex and the centroid of the other vertices, establishing a new favourable vertex. So, a new Simplex is generated that is different from the previous by the new vertex.

The Simplex method can be as follows:

a). fixed-size sequential simplex method;

b). variable-size simplex algorithm.

The fixed-size sequential simplex method is an algorithm consisting simply of reflection rules. This method is slow and a local optimum could be attained instead of general optima.

The variable-size simplex algorithm is a method consisting of reflection, expansion and concentration rules. If a vertex lies outside the boundaries of

one or more of the factors, a very undesirable response is assigned to that vertex. The simplex will then force back inside the boundaries. The simplex is halted when the step size becomes less than some predetermined value or when the differences in response approach the value of the experimental uncertainty, or when adequate response has been achieved.

4.2.4. Prisma Method

This method was introduced by Nyiredy and co-authors to optimize the solvent system in TLC /65-68/. For the selection of suitable solvents the first experiments are carried out on TLC plates in unsaturated chambers with 10 solvents, chosen from the different selectivity groups of Snyder /19/. After these experiments, the solvent strength has either to be reduced or increased so that the Rf values of the substances are distributed in the range 0.2 - 0.8. If the substances migrate into the upper third of the plate the solvent strength has to be reduced by dilution with hexane (solvent strength = 0). If the substances remain in the lower third of the plate the solvent strength has to be increased by addition of water or acetic acid. A similar procedure is followed in the reversed-phase mode, except that solvent selection is limited to water-miscible solvents.

Between two and five solvents can be selected for construction of the Prisma model. Modifiers can be added to improve the separation and reduce tailing. Modifiers are generally used in low and constant concentration so that their influence on solvent strength can be neglected. The Prisma model is a three-dimensional geometrical design that correlates the solvent strength with the selectivity of the mobile phase /69/. The lengths of the edges of the prism correspond to the solvent strength of the solvents. Since different solvents usually have different solvent strength, the lengths of the edges of the prism are unequal and the top plane of the prism will not be parallel and congruous with its base. If the prism is intersected parallel to its base at the height of its shortest edge, the lower part gives a regular prism. So, the model consists of three parts: the base or platform representing the modifier; the regular part of the prism; and the irregular top part of the prism (frustum). The upper frustum of the model is used for mobile phase optimization of polar compounds in normal phase chromatography, while the regular part is used for the separation of nonpolar and moderately polar substances. For reversed-phase chromatography, the regular part of the prism is used to optimize the separation of both polar and nonpolar substances.

For polar compounds optimization is always started on the top irregular triangle of the model, either within the triangle when three solvents are selected, or along one side, when two solvents are selected. Each solvent composition on the surface of the triangle can be described by three-coordinate selectivity points. Optimization is commenced by selecting solvent combinations corresponding to the centre point and three other points close to the apexes of the triangle. If the obtaining separation is insufficient other selectivity points are tested around the solvent combination that gave the best separation. On changing the selectivity points on the top triangle the solvent strength changes as well, especially when the strengths of the solvents used to construct the prism differ considerably. The strength of the solvent should be adjusted with the adjusting solvent to maintain the separation in the optimum R_f range. It may also be advisable to change the selectivity points by small increments if regular step sizes cause large changes in resolution.

The regular centre portion of the prism is used to optimize the mobile phase composition for the separation of nonpolar and moderately polar samples. The initial solvent composition corresponds to the centre of the triangular top face of the regular prism. This composition is then diluted to bring all sample components into the R_f range 0.2 - 0.8. At this solvent strength three more chromatograms are run corresponding to the selectivity points close to the apexes of the triangle. These initial runs are then used to choose the selectivity points for further chromatograms until the best solvent composition are located.

Nyiredy and co-workers /70/ have described the vertical and horizontal correlation between the hR_f values of nonpolar compounds and the selectivity points at different constant levels of the solvent strength in saturated chambers. The vertical correlation and the horizontal correlation are given by the following equation:

$$\ln hR_f = d(S_T) + e \tag{13}$$

$$hR_f = a(P_s)^2 + b(P_s) + c \tag{14}$$

4.2.5. Overlapping Resolution Mapping Scheme (ORM)

The introduction of this method by Glajch and co-workers /71/ is one of the most important additions to the literature of mobile phase optimization. It involves the generation of coefficients for a quadratic equation containing between six and ten terms that allows the generation of a chromatographic response surface for a mixture of solvents.

The ORM scheme is an interpretative optimization method in which the extent of chromatographic separation is predicted indirectly from the retention behaviour of the individual solutes /72/. For the optimization of eluent mixtures with up to quaternary compositions, only seven experiments need to be carried out according to the Snyder selectivity triangle before one can locate the optimum conditions /19/.

A schematic diagram of the ORM scheme follows: /73/:

setting criteria ·
↓
selection of appropriate eluotropic strength ·
↓
define the three vertices of the solvent selectivity triangle ·
↓
perform set of seven experiments ·
↓
calculate resolution for each pair of peaks
↓
determine coefficients for second order polynomial
↓
· plot diagrams for each pair of peaks ·
↓
superimpose individual diagrams to obtain ORM diagram

The resolution, R_s, could be calculated using a second-order polynomial:

$$R_s = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \qquad (15)$$

where a_i are coefficients and x_i, the volume fractions of the mobile phases.

The values of resolution were used to construct the diagrams for all peak pairs. The individual resolution plots were then superimposed to give an ORM diagram.

The ORM optimization scheme is a rapid and versatile method. Optimum separation can be achieved without much difficulty even when quaternary mobile phases are considered, because only seven different mobile phase systems need to be examined in order to obtain the necessary data for the resolution plots.

4.3. Mathematical Methods

4.3.1. Numerical Analysis Method (Factorial Designs)

Selection of optimal solvent composition in a given binary system of selected selectivity can be carried out by conducting at least three experiments, with one experiment at each of three different solvent compositions /7/. The different responses, which are expressed as resolution, are fitted to a quadratic polynomial:

$$R_s = a + bY_1 + cY_1^2 \tag{16}$$

where Y_1 is the composition of one component of the solvent. The optimum composition is obtained by setting the first derivative of the fitted equation equal to zero

$$Y_{1\,optimal} = \frac{-b}{2}c \tag{17}$$

This method was applied by Turina et al. /74/ to optimize the resolution of a lipid mixture in a two-component mixture, where the amount of one component is changed and the amount of other is kept constant.

To simplify calculations, the three compositions of component 1, Y_0, Y_1 and Y_2, are varied in a fixed amount, ΔY, with respect to a chosen value:

$$Y_0 = Y_0; \quad Y_1 = Y_0 + \Delta Y; \quad Y_2 = Y_0 + 2\Delta Y \tag{18}$$

The resolution values, R_s, obtained using the three selected concentrations are fitted to the polynomial equation (16), which is written in terms of differences:

$$R_s = f(Z) = f_0 + Z\Delta f_0 + Z(Z-1)\frac{\Delta^2 f_0}{2} \tag{19}$$

with the first derivative equal to:

$$f'(Z) = \Delta f_0 + Z\Delta^2 f_0 - \frac{\Delta^2 f_0}{2} \quad (20)$$

where:

$$Z_i = \frac{Y_i - Y_0}{\Delta Y}; \quad \Delta f_0 = (R_s)_1 - (R_s)_0; \quad \Delta^2 f_0 = (R_s)_2 - 2(R_s)_1 + (R_s)_0 \quad (21)$$

The optimum value of Z is obtained, making equation (20) equal to zero, that is:

$$Z_{optimum} = \frac{(R_s)_2 - 4(R_s)_1 + 3(R_s)_0}{2(R_s)_2 - 4(R_s)_1 + 2(R_s)_0} = -\frac{\Delta f_0}{2\Delta^2 f_0} \quad (22)$$

The optimum concentration is finally obtained, transforming this result to a concentration scale:

$$Y_{optimum} = Y_0 + \Delta Y Z_{optimum} \quad (23)$$

This method may be extended to ternary solvent systems by maintaining one volume constant and varying the other two volumes. If there are no interactions between solvent components the resolution is approximately expressed by:

$$R_s = a + bX + cY + dX^2 + eY^2 \quad (24)$$

To apply a similar mathematical treatment, five experiments are necessary /7/.

If there is interaction between the components, the model must be modified and further experiments are required. For example, a five-component solvent system has been studied, in which two of the components have interactions. In this case, the volume of the interacting pair was independently varied, and the volumes of the other three were kept constant. In this case, the resolution is approximated by the model:

$$R_s = a + bX + cY + dX^2 + eY^2 + fXY \quad (25)$$

Nine experiments must be conducted to obtain the optimum and the final result is obtained by a graphical method.

4.3.2. Numerical Taxonomy Technique

The taxonomy method has been used originally in biological research and does allow a formal classification. This technique is somewhat intuitive and tends to be subjective, so that taxonomy has been said to be an art rather than a science.

A more quantitative approach, numerical taxonomy, has been developed relatively recently. This method uses a variety of related mathematical techniques to order the classification of individuals in groups, or groups of individuals in larger groups. The units to be classified are generally called operational taxonomic units (OUT). The OUT in the chromatographic problem is the solvent / stationary phase system /75, 76/.

The fundamental idea of numerical taxonomy is to attach numerical values to a number of characteristics for each of the OUT's and to compare these values to discover similarities. The OUT's can then be classified according to their resemblance. In the chromatographic problem, the characteristics will be migration parameters such as R_f values, Kovats indices, etc., for a number of standard substances or for the compounds composing the group that has to be separated.

In general, the procedure consists of three steps:

1). The data, x_{ij}, are recorded in an (i x j) matrix with i = n characteristics and $j = j_{max}$ OUT's. In chromatography, n is limited to the total number of substances for which a separation is being investigated.
2). The next step is to compare each OUT with each other OUT and to record this comparison as a similarity value. Many kinds of different similarity values have been proposed in numerical taxonomy and one of the most used is a measure of distance.
3). The third step is to group together the OUT's with the largest similarity and many methods to achieve this has been proposed.

The combination of the numerical taxonomy classification and calculation of the information content is an example of trends in analytical chemistry.

BIBLIOGRAPHY

1. H.J.G. Debets, *J. Liq. Chromatogr.*, 8 (15), 2725 (1985).
2. P.J. Schoenmakers, *Optimization of Chromatographic Selectivity*, Elsevier, Amsterdam, 1986.
3 J.C. Berridge, *J.Chromatogr.*, **485**, 3 (1989)
4. Sz. Nyiredy, K. Dallenbach - Toelke and O.J. Sticher, *J. Planar Chromatogr.*, 1 (Dec), 336 (1988).
5 A.G. Howard and L.A. Bonicke, *Anal. Chim. Acta*, **223**, 411 (1989).
6. E. Heilweil, chap. 3 in : *Mobile Phase Design and Optimization*, 37-49, 1985.
7. S. Gocan, chap. 8 in *Modern Thin Layer Chromatography*, ed. Nelu Grinberg, 435, 1990
8. G. Guiochon, F. Bressolle and A.J. Siouffi, *Chromatogr. Sci.*, **17**, 368 (1979).
9. D.N. Deming and M.L.H. Turoff, *Anal. Chem.*, **50**, 546 (1978).
10. C. Liteanu, S. Gocan, T. Hodisan, H.Nascu, *Cromatografia de Lichide*, Ed. Stiintifica , Bucuresti, 1974.
II. E. Jercan, *Analiza Cromatografica*, Ed. Academiei, Bucuresti, 1982.
12. L.R. Snyder, *Principles of Adsorption Chromatography*, Edward Arnold, London and Marcel Dekker, New York, 1968.
13. C. Liteanu, S. Gocan, *Separatologie Analitica*, Ed. Dacia, Cluj-Napoca, 1981.
14. L.R. Snyder, *J. Chromatogr.*, 8, 178 (1962).
15. E. Heftmann, *Chromatography*, Reinhold Publishing Corporation, New York, 1967.
16. D.L. Saunders, *Anal. Chem.*, 46, 470(1974).
17. L. Rohrschneider, *J. Chromatogr.*, **22**, 6(1966).
18. L.R. Snyder, *J. Chromatogr.*, **92**, 223 (1974).
19. L.R. Snyder, *J. Chromatogr. Sci.*, 16, 223 (1978).
20. L. Rohrschneider, *Anal. Chem.*, **45**, 1241(1973).
21. P.E. Autle, *Chromatographia*, **15**, 277 (1982).
22. J.L. Glajch, J.J. Kirkland, L.R. Snyder, *J. Chromatogr.*, **238**, 269 (1982).
23. L.R. Snyder, J.L.Glajch, *J. Chromatogr.*, **214**, 1(1981).
24. L.R. Snyder, J.L. Glajch, J.J. Kirkland, *J. Chromatogr.*, **218**, 299 (1981).
25. B.P.Johnson, M.G.Khaledi, J.G.Dorsey, *Anal. Chem.*, 58, 2354 (1986)

26. L.R. Snyder, J.W. Dolan and J.R. Gant, *J. Chromatogr.*, **165**, 3 (1979).
27. A.F.M. Barton, *Chem. Rev.*, **75**, 731(1975).
28. B.L. Karger, L.R. Snyder and C. Eon, *J. Chromatogr.*, **125**, 71 (1976).
29. S. Gocan, chap. 3 in *Modern Thin Layer Chromatography*, ed. Nelu Grinberg, 156, 1990.
30. J.A. Dean, *Chemical Separation*, Van Nostrand Reinhold, New York, **188**, 1969.
31. R.P.W. Scott, *J. Chromatogr.*, **122**, 35 (1976).
32. G. Rouser, *J. Chromatogr.* Sci., **11**, 60 (1973).
33. G. Rouser, *J. Chromatogr.* Sci., **8**, 619 (1970).
34. A.H.Stead, R Gill, T.Wright, J.P.Gibbs and A.C.Moffat, *Analyst*, **107**, 1106 (1982).
35. L. Lepri, P.G. Desideri and R. Mascherini, *J. Chromatogr.*, 70, 212 (1972).
36. B.L. Karger, L.R. Snyder and C. Horvath, *An Introduction to Separation Science*, Wiley, New York, 1973.
37. I. Malinowska, J.K. Rozilo, *J. Planar Chromatogr.*, **6**, 452 (1993).
38. D. Nurok, *Chem.Rev.*, **89**, 363-375 (1989).
39. R.P.W. Scott, *J. Chromatogr. Sci.*, **18**, 297 (1980).
40. H. Kenker, B.G. Balder, M.P. van Berkel, H.M. Hamstra-Spikkers, J.A. Olthof and J.C. Tegelbeckers, *Proceedings of the International Symposium on Instrumental TLC / Planar Chromatography*, Brighton, Sussex, U.K., 1989; 105.
41. I. Malinowska, J.K. Rozylo, A.Gumieniak, *J. Planar Chromatogr.*, **8** (1), 23 (1995).
42. H.Nascu, T.Hodisan, Claudia Cimpoiu, *Stud. Univ. B-B, Chemia,* **XXXIX** (1-2), 167 (1994).
43. T. Hodisan, H. Nascu, Claudia Cimpoiu, I. Hopârtean, *Rev. Roum. Chim.*, 41 (1-2), ss (1996).
44. S.L. Morgan, S.N. Deming, *Sep. Purif. Methods*, **5**, 333 (1976).
45. R. Kaiser, *Euroanalysis IInd Conference*, Budapest, **43** (1975).
46. J. Souto, A.G. de Valesi, *J. Chromatogr.*, 46, 274 (1970).
47. D.L. Massart, *J. Chromatogr.*, 79, 157 (1973).
48. D.L. Massart, A. Dijkstra, R. Smits, *Euroanalysis IInd. Conference,* Budapest, 52 (1975).
49. S. Gocan, M. Mihaly, *Stud. Univ. B-B, Chemia*, **1**, 18(1981).
50. H. Nascu, C. Sârbu, Elena Moraru, T. Hodisan, *Rev. Chim.*, **33** (6), 550 (1982).
51. C. Sârbu, H. Nascu, *Rev. Chim.*, 41(3), 271(1990).

52. R. Smits, C. Vanroelen, D.L. Massart, *Fres. Z. Anal. Chem.*, **273**, 1(1975).
53. S.L. Morgan, S.N. Deming, *J. Chromatogr.*, **112**, 267 (1975).
54. W.A. Spencer, L.B. Rogers, *Anal. Chem.*, **52**, 950 (1982).
55. P.J. Schoenmakers, A.C.J.H. Drouen, H.A.H. Billiet, L. Galan, *Chromatographia*, **15**, 688 (1982).
56. F. Doudi, Y.D. Kahie, P. Reschiglian, C. Bighi, G.P. Cartoni, *Chromatographia*, **23**, 844(1987).
57. F.V. Warren Jr., C.H. Phoebe Jr., M. Webb, A. Weston, B.A. Bidlingmeyer, *Int. Lab.*, **5** (Jul/Aug), 14 (1991).
58. J.C. Berridge, *Chromatographia*, **146**, 172 (1982).
59. A.G. Wright, A.F. Fell, and J.C. Berridge, *Chromatographia*, **24**, 335 (1987).
60. R.J. Laub, J.H. Purnell, *J. Chromatogr.*, 112, 71(1975).
61. D. Nurok, J.M. Richard, *Anal. Chem.*, 53, 563 (1981).
62. D. Nurok, R.M. Becker, M.J. Richard, P.D. Cunningham, W.B. Gorman and C.L. Bush, *J. High Res. Chromatogr. Chromatogr. Commun.*,**5**, 373 (1982).
63. D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte and L. Kaufman, *Chemometrics: A Textbook*, Elsevier New York, NY, USA, 1988.
64. S.N. Deming, S.L. Morgan, *Anal. Chem.*, **45** (3), 278 (1973).
65. .K. Dallenbach-Toelke, Sy. Nyiredy, B. Meier, O. Sticher, *J. Chromatogr.*, **365**, 63 (1986).
66. C.F. Poole, S.K. Poole, *Chromatography Today*, Elsevier, USA, 1991.
67. Sz. Nyiredy, B. Meier, C.A. Erdelmeier and O. Sticher, *J. High Res. Chromatogr. Chromatogr. Commun.*, 8, 186 (1985).
68. K. Dallenbach-Toelke, Sz. Nyiredy, S.Y. Meszaros and O. Sticher, *J. High Res. Chromatogr. Chromatogr. Commun.*, **10**, 362 (1987).
69. Sz. Nyiredy, K. Dallenbach-Toelke and O. Sticher, *J. Liq. Chromatogr.*, **12**, 95 (1989).
70. Sz. Nyiredy, Zs. Fater, *J. Planar Chromatogr.*, 8 (5), 341 (1995).
71. J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, *J. Chromatogr.*, 199, 57 (1980).
72. J.L. Glajch and J.J. Kirkland, *Anal. Chem.*, **55** (2), 319 A (1983).
73. S.F.Y. Li, H.K. Lee, C.P. Ong, *J. Chromatogr.*, **506**, 245(1990).
74. S. Turina, M. Trbojevic, M. Kastelan-Macan, *Anal. Chem.*, **46** (8), 988 (1974).
75. D.L. Massart, H.De Clercq, *Anal. Chem.*, **46** (13), 1988 (1974).
76. R.S. Henly, *J. Chromatogr. Sci.*, **11**, 221(1973).