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Review

Some aspects of optimization in planar chromatography

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ABSTRACT

A survey of published optimization procedures in thin-layer chromatography (TLC) is presented. In one-dimensional TLC the mobile phase selection is performed through either computerized or non-computerized methods. Most of the latter methods are similar to those utilized in high-performance liquid chromatography and have been advocated for planar chromatography (simplex, overlapping resolution map). Resolution-based criteria have been criticized and others are proposed. Some procedures are predictive whereas others are not, such as principal component analysis. In two-dimensional TLC the aim is to find two systems exhibiting the least correlation. In this respect no attempt has been made to optimize the stationary phase.

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1. INTRODUCTION

It is often considered that efficiencies achieved in planar chromatography (PC) are low in comparison with high-performance liquid chromatography (HPLC) and, as

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a consequence, efforts to optimize PC are not numerous. This is emphasized by the fact that many runs can be carried out within a short period of time in classical PC [thin-layer chromatography (TLC)], thus supplying the analyst with a large amount of data. The best conditions for separation are determined by trial and error, relying on the expertise of the chromatographer.

Fortunately, PC has evolved toward an instrumentalized technique capable of producing a high degree of sensitivity, selectivity and efficiency with reliable reproducibility.

In the more general case, the goal of the optimization process must be to improve the separation between all the peaks representing the individual components of a mixture in order to obtain a chromatogram in which each peak will correspond to one and only one component. This goal is achieved by adjusting a set of experimental factors. Optimization in PC is important as the length of the plate and the time of analysis are fixed and there is no way to couple plates in series. Moreover, the unique feature of PC is its two-dimensional capability and it has been demonstrated by Guiochon and co-workers [1,2] that the peak capacity in two-dimensional TLC is much larger than those obtained with the best HPLC columns.

Optimization involves three steps: (i) definition of the criterion, (ii) definition of the parameter space and (iii) logical procedure.

We shall not consider the "one-variable-at-a-time" (univariate) methods to focus on multivariate optimization. Optimization of one variable is performed on discrete variables such as particle size, pH and ionic strength. Multivariate methods deal with related variables such as the amount of each type of solvent used in the mobile phase. These are related variables as the sum of all solvents must total 100%. The first paper on the topic was from Guiochon *et al.* [3], who determined the relationships between development length, analysis time and particle diameter to achieve the best performances (see, *e.g.*, Fig. 17 in ref. 3).

At present the analyst selects one stationary phase and searches for the optimum solvent. Although many ways of changing selectivity in chromatography are possible, the most powerful is to change the composition of the mobile phase. In HPLC the majority of separations are carried out with alkyl-bonded phases. Conversely, bare silica is still widely used in PC. Smith and Cooper [4] described a stationary phase selectivity triangle in which only polar bonded phases are displayed. Mixtures of stationary phases are unusual. One attempt was made by Righezza and Siouffi [5], who demonstrated that it works well with non-polar solutes whereas peak broadening occurs with polar solutes [6]. Published optimization procedures deal with the selection of the solvent, which can be a time-consuming operation.

We shall distinguish one-dimensional classical TLC from more advanced techniques and computerized methods from non-computerized methods. It does not seem that special optimization procedures devoted to forced flow or high-pressure PC have been published.

2. ONE-DIMENSIONAL PLANAR CHROMATOGRAPHY

2.1. Grid search procedures

A graphical method has recently been published by Issaq and Seburn [7]. The system is based on a plot of observed R_F values *versus* the composition of a binary

mobile phase. The method is said to be simple and has been applied to both normal- and reversed-phase TLC. Two primary solvents, A and B, are selected and five data from five mixtures are required to plot R_F values *versus* mobile phase composition. This procedure is only experimental. Unfortunately, A and B are not single solvents, *e.g.*, A is acetonitrile–water and B is methanol–water in a reversed-phase system. The selection of primary solvents A and B is not straightforward and it does not seem that this procedure can be used with complex mixtures.

Oscik-Mendyk [8] proposed displaying the R_F data on a Gibbs triangle in the form of isolines, *i.e.*, lines connecting the points corresponding to the same R_F values. These lines are not parallel and regions where R_{F_1}/R_{F_2} is higher permits the selection of the appropriate solvent. It is particularly useful with ternary mobile phases but it is time consuming as a large number of experiments are required and there is no means to predict the retention behaviour.

The PRISMA model developed by Nyiredy and co-workers [9–11] is a three-dimensional model correlating the solvent strength and the selectivity of mobile phases. Silica is used as the stationary phase and solvent selection is performed according to Snyder's solvent classification [12]. Preliminary experiments are carried out with different solvents from the eight groups of the classification. The solvent strength has to be reduced or increased so that the substance zones are distributed between $R_F = 0.2$ and 0.8. For this purpose, when R_R values are too high or too low modifiers (hexane or water) are added. Water saturation can be used. The PRISMA model has three parts: an irregular frustum, a regular middle part and a platform (Fig. 1). The three top corners of the model represent the selected three individual solvents which can be diluted with hexane (eluotropic strength = 0). The solvent strength is represented by the height of the prism, points along the edges stand for combination of two solvents, points on the sides for combination of three and points in the interior of the prism for mixtures of four solvents. The prism is similar to that proposed by Glajch and Kirkland [13] for experimental design approach for gradient elution.

The PRISMA model includes all combinations of one to five solvents for the separation of compounds from low to high polarity. With non-polar samples the initial solvent composition corresponds to the centre of the triangular top face of the regular prism. This composition is diluted with hexane to bring solutes into the convenient R_F range. The solvent strength is maintained and a further three chromatograms are run at solvent compositions corresponding to selectivity points near the apices of the triangle. From these initial runs further chromatograms with different compositions are obtained until the best solvent mixture is reached. With polar samples the upper face of the frustum is utilized and the optimization proceeds in a very similar way. The last step is the selection of the appropriate development mode (linear, circular, anticircular, etc.). The PRISMA is a structured trial-and-error method. The TLC system is not computerized. In our opinion, the PRISMA is very powerful for the selection of mobile phase in over-pressurized layer chromatography. However, when four or five components are selected as the best mobile phase the TLC experiments may be tedious because of demixing.

2.2. *Computer-assisted methods*

These can be divided into two categories: simultaneous and sequential methods.

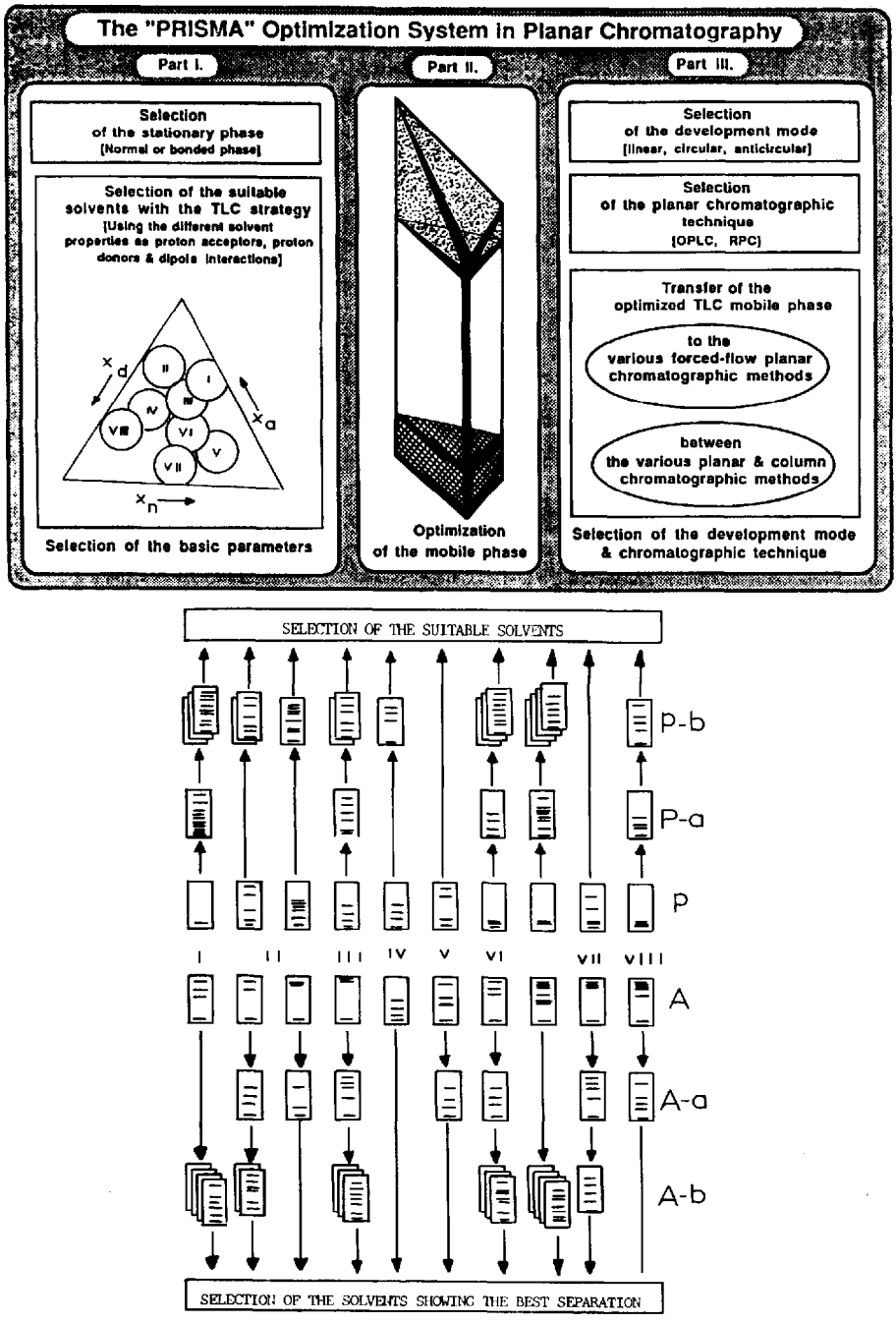

Fig. 1. Illustration of the PRISMA optimization procedure (reprinted from ref. 11, with permission).

In every case a response criterion summarizes the quality of the chromatogram in a single numerical value. In many instances binary eluents are utilized, which permit the use of the linear relationship between R_M (or log k') and log X_s, where X_s, is the mole fraction of the component of greater eluting strength. In normal-phase chromatography the binary eluent is formed with an apolar diluent (*e.g.*, hexane) and a polar modifier; in the reversed-phase mode X_s is the proportion of organic modifier in the water–organic solvent mixture.

$$R_M = a \log X_s + b \tag{1}$$

This relationship holds true in both systems; a and b are constants characteristic of a given compound. One important feature is that the comparison of theoretically equieluotropic mixtures shows that this is only true for a given reference solute and there is an individual contribution from the solute molecular structure [14]. This means that two different binary mixtures of the same calculated eluotropic strength will yield different R_F values for different solutes on the same stationary phase. This precludes the *a priori* selection of isoeluotropic mixtures when dealing with samples containing very different species.

Nurok and Richard [15] used the above linear relationship to calculate R_F values for pairs of solutes at different mole fractions of a binary mixture of solvents to determine the ΔR_F. Plots of ΔR_F *versus* the mole fraction of the polar modifier in the normal-phase mode exhibit a maximum. Moreover, a plot of ΔR_F *versus* solvent polarity parameter exhibits another ΔR_F maximum corresponding to another binary mixture (Fig. 2). This $\Delta R_{F_{max}}$ criterion is used in many schemes as it is related to capacity factors through

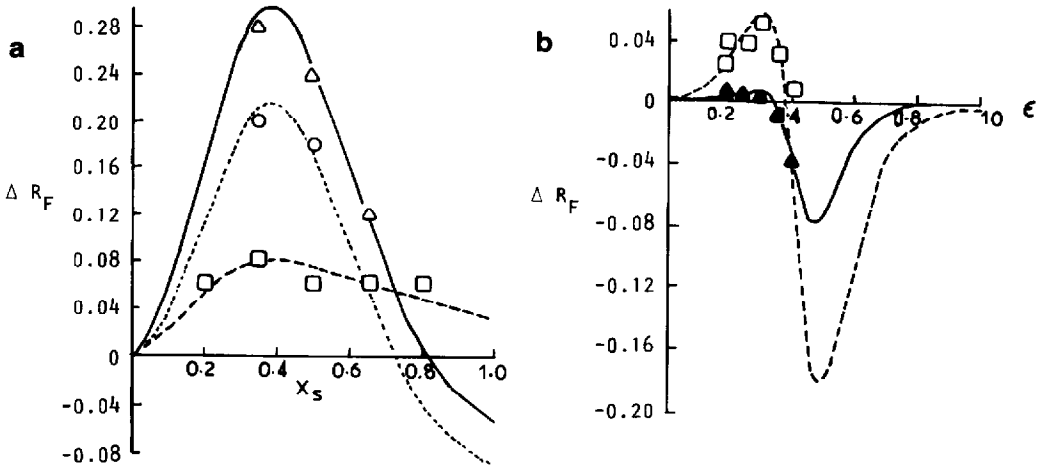

Fig. 2. (a) Plot of ΔR_F *vs.* X_s, the mole fraction of the polar modifier in an acetone–cyclohexane binary mixture. △, ○, □, Experimentally determined ΔR_F values. Pairs of solutes are as follows: solid line, hydroquinone–*o*-cresol; dotted line, hydroquinone–phenol; dashed line, phenol–*o*-cresol. (b) Plots of ΔR_F *vs.* the solvent polarity parameter for a chloroform–carbon tetrachloride binary mixture. Solutes are dyes: solid lines, butter yellow/sudan green; dashed line, oil orange/sudan green. (Reprinted from ref. 15, with permission.)

$$\Delta R_F = \frac{k'_j - k'_i}{(1 + k'_j)(1 + k'_i)} \tag{2}$$

2.3. Computerized method through a data base

This procedure was proposed by Matyska and co-workers [16,17]. Two experiments are required to determine constants a and b and many data were gathered by the authors. The optimization program has five steps: input section where names (as codes) of solutes are introduced, computation of R_F values, sorting and calculation of R_F, choice of the best eluent composition corresponding to the largest value for minimum R_F and output section. R_F values in the range 0.3–0.4 are considered when the volume percentage of polar modifier is low. The program is written in BASIC. The aim is to analyse toxic substances and rapidly select a solvent that differentiates two solutes exhibiting same retention in one system.

From eqn. 1 we can write for two solutes i and j

$$\log \alpha = \log X_s (a_j - a_i) + (b_j - b_i) \tag{3}$$

and

$$\frac{\mathrm{dlog}\ \alpha}{\mathrm{dlog}\ X_s} = a_j - a_i \tag{4}$$

when $a_j > a_i$ the selectivity will increase with increasing amount of the modifier, and when $a_j < a_i$ the reverse is observed.

A different equation was proposed by Oscik [18] to relate the R_M value of a chromatographed substance using a multi-component mobile phase to R_M values of the same substance in a single mobile phase. However, it requires the determination of excess adsorption isotherms and its application looks tedious.

2.4. Window diagrams

This technique was introduced in gas chromatography by Laub and Purnell [19]. It has recently been advocated for the selection of mobile phases in HPTLC [20]. It is based on the same equation as eqn. 1 but rewritten in the form

$$R_F = \frac{1}{1 + \exp(a \ln X_s + b)} \tag{5}$$

The separation between two spots is

$$\Delta R_F = R_{F_i} - R_{F_j} = \frac{1}{1 + \exp(a_i \ln X_s + b_i)} - \frac{1}{1 + \exp(a_j \ln X_s + b_j)} \tag{6}$$

A window diagram is used to plot the ΔR_F *versus* mobile phase composition. The maxima at the top of the window represent the mobile phase composition giving the best separation for the least separated pair (Fig. 3). A perpendicular from the tallest window to the abscissa identifies the optimum composition. This procedure requires the same number of experiments as the previous one. The location of peak cross-overs is easier.

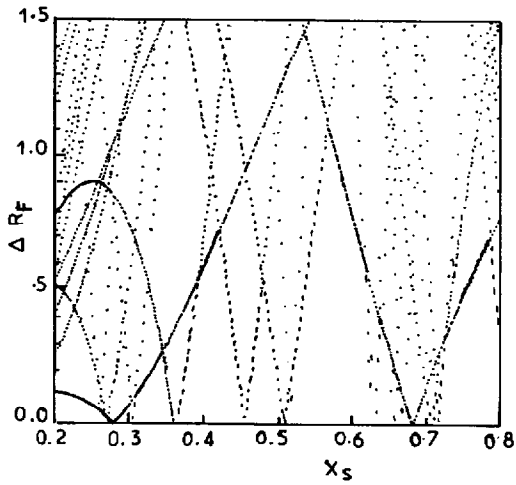

Fig. 3. Typical window diagram utilized for determining the optimum solvent composition in the separation of pesticides (reprinted from ref. 27, with permission).

2.5. Sequential optimization procedures

The simplex method is based on the principle of stepwise movement toward the set goal with simultaneous changes of several variables. The simplex itself is a geometric shape with one more vertex than the number of variables under study. In the optimization each vertex corresponds to a set of operating parameters and it is necessary to run a chromatogram with these conditions. The quality of the separation achieved is assessed for each vertex and ranked from best to worst. The worst separation is discarded and a new vertex is constructed by reflecting it through the plane joining the remaining vertex. A modified simplex with expansion or contraction is more convenient for TLC.

Turina [21] proposed the following course with simplex: input data, simplex design, experiments, testing the experiments, rejection of the worst vertex, centroid, new vertex, new experiment, new simplex. There are several disadvantages to the sequential approach: the optimum that is located is not *a priori* the global optimum and is dependent on the choice of the response function and the initial setting of the experimental variables. For HPLC Berridge [22] proposed the chromatographic response function, $CRF = \Sigma \ln (P_i)$, where P_i is the peak separation.

In the separation of three dyes, Sabate and Tomas [23] considered that the distance between spots and the response is

$$S = \frac{4\,(R_{F_3} - R_{F_2})(R_{F_2} - R_{F_1})}{(R_{F_3} - R_{F_1})} \tag{7}$$

where the denominator is the distance between the highest and lowest spots. Some rules were given to transform the "rigid" simplex and accelerate the location of the optimum.

According to De Spiegeleer *et al.* [24], comparison of literature data with

a resolution-based criterion is tedious or even not possible owing to the lack of information on the spot widths. They proposed a multi-peak response function:

$$\frac{[hR_F(\max) - hR_F(n)][hR_F(l) - hR_F(\min)] \prod_{i=1}^{n-1} (hR_F(i+1) - hR_F(i))}{\{[hR_F(\max) - hR_F(\min)]/(n+1)\}^{n+1}} \cdot 100\% \quad (8)$$

The criterion is expressed as a percentage (0–100%) and it has an intrinsic and universal meaning which permits the comparison of published separations. The idea is that the optimum solvent is the one that yields a chromatogram where all the spots lie at equal distances from each other and from chosen boundaries. In this mode the R_F range may be selected. According to the authors [24], eighteen vertices are required to reach a 99.1% response in order to select a suitable solvent for the complete separation of platinum complexes.

2.6. Statistical mixture design with isoresponse curves

The overlapping resolution map (ORM) technique has been successfully introduced in HPLC by Glajch *et al.* [25]. In this procedure three selectivity-adjusting solvents for either mode [methanol, tetrahydrofuran (THF) and acetonitrile for the reversed-phase (RP) mode and diethyl ether, methylene chloride and chloroform for the normal-phase (NP) mode] plus a strength-adjusting solvent for either mode (water for RP, hexane for NP) require the use of a total of four solvents to carry out the selectivity optimization. The three apices of the selectivity triangle represent isoeluotropic binary mixtures of the diluent and the modifier. A series of 7–10 experimental runs are necessary to calculate the coefficients of the response function. The chromatographic optimization function (COF) describes the resolution between pairs of compounds inside the triangle (the factor space). Overlapping of these maps permits the selection of the optimum mobile phase. It must be pointed out that the selectivity triangle can be used with any three parameters. In this way, Tecklenburg *et al.* [26] plotted plate length, binary solvent composition and analysis time to optimize the TLC separation of fifteen steroids. A similar plot has been published recently for the separation of thirteen pesticides [27].

The ORM technique has been adapted to HPTLC by Issaq *et al.* [28] for the separation of four naphthalene derivatives on C_{18} plates. Bayne and Ma [29] used the resolution function

$$R_s(j, j+1) = 2(D_{j+1} - D_j)/W_j + W_{j+1} \quad (9)$$

where D_j and D_{j+1} are migration distances of two adjacent spots and W_j and W_{j+1} are the spot diameters. As an NP system with propylamino-bonded silica gel plates was utilized, the diluent was hexane and benzene (group 7 of Snyder's selectivity triangle), chloroform (group 8) and THF (group 3) were the selected modifiers. As usual in HPLC, a Scheffe's second-order polynomial was used for predicting the resolution of each pair of adjacent spots:

$$Y = A_1X_1 + A_2X_2 + A_3X_3 + A_1A_2X_1X_2 + A_1A_3X_1X_3 + A_2A_3X_2X_3 \quad (10)$$

where Y is the response, X_i the proportions of solvents 1, 2 and 3 with $X_1 + X_2 + X_3 = 1$, A_i are the expected responses for pure components and A_iA_j are synergistic coefficients. Ten experiments were carried out for the eleven dyes of interest and the optimum was located. The authors compared the results with those obtained with the ideal separation (*IS*) function they advocated previously.

Bayne and Ma [30] used statistical mixture experiments to find the best solvent system to separate twelve structurally related benzo[*a*]pyrene metabolites. Starting with n solvents an $n - 1$ dimensional simplex was defined. Preliminary tests were carried out to estimate the solvent strength capable of yielding R_F values of the solutes within a selected range. Some regions were of no interest and truncations were performed, which yielded a polyhedron. As the authors considered five solvents, the domain was a truncated four-dimensional simplex. The separation response model had fifteen coefficients and five additional runs were performed to estimate the experimental error. A total of 20 runs were required. Response functions were tested and four were discarded [maximizing overall distances, minimizing inverse distances, maximizing a function based on the difference ln $(R_{F_i} - R_{F_j})$, maximizing adjoining distances]. All these functions exhibited failures when eccentric spots were observed. The ideal response function is the sum of an ideal spacing term and an ideal spread term. The ideal separation of q components would have R_F values equally spaced on the unit interval (0–1). The ideal value for the jth ordered R_F value would be $(j - 1)/(q - 1)$. The idea is similar to that of De Spiegeleer *et al.* [24]. To account for spreading, Bayne and Ma [32] suggested the use of the standardized fourth central moment of the R_F values:

$$b_2 = (M_4/M_2)^2 \tag{11}$$

For q components equally spaced

$$B_2 = 3(3q^2 - 7)/[5(q - 1)(q + 1)] \tag{12}$$

and

$$IS = \left(\sum [(R_F)_j - (j - 1)/(q - 1)]^2 + (b_2 - B_2)^2\right)^{1/2} \tag{13}$$

The aim is to reach the lowest value of *IS*. The search considered 41 469 cases!

ORM and *IS* methods resulted in different mobile phase compositions. From the authors' conclusion, the ORM method focuses on the separation of the nearest pair of spots whereas the *IS* approach maximizes the overall separation of all spots.

2.7. Statistical approach

Computer-assisted multivariate techniques offer the possibility of evaluating all retention data simultaneously. Principal component analysis (PCA) provides an approximation of a data matrix. In PCA one considers each row in the data matrix to be a point in a multi-dimensional space with coordinates defined by the values corresponding to the appropriate n columns in the data matrix. Each solute is treated as a point in a space defined by its retention coordinates along the different solvent composition axes. The PCA extracts axes (or eigenvectors) that best span the data

matrix. The first eigenvector is computed such as the sum of the magnitudes of the projections of all points on that vector is a maximum. The second eigenvector is chosen orthogonal to the first so that as much of the remaining variation lies along this vector. The data matrix is thus decomposed into two matrices, the row cofactor matrix and the column cofactor matrix.

Cserhati and co-workers [31–33] used PCA and spectral mapping techniques to optimize the selectivity of mixed packings in amino acid analysis. Glycine and glutamine give rise to severely overlapped zones [34] and from PCA results together with target transformation quantification, capabilities were estimated and compared with the Kalman filter. Two recent papers examined the separation of steroids on silica gel with fifteen mobile phases [35] and on both silica gel and RP-18 plates [36]. It permits the mobile phases to be selected that provide the highest selectivity.

A Plackett–Burman factorial design at two levels has been proposed by Olsson *et al.* [37] in lipid analysis. In this mode a plot of principal properties of organic solvents was drawn in the form of a quadrant where the axes are linear combinations of those physical variables. Two solvents were chosen from each of the quadrants, one near the origin (low level, −) and one far from the origin (+ level). These factors were varied together with chromatographic conditions. From this screening procedure a principal component map of the design space is drawn, followed by a multivariate regression model.

2.8. Gradient in TLC

Markowski [38] has developed equations to predict R_{F_g}, *i.e.*, the R_F value of a solute chromatographed under stepwise gradient conditions. A binary mobile phase is utilized in such a way that the total volume introduced in the layer is equal to the void volume. A relative resolution is selected as a criterion where the distance between spots and spreading of a solute spot in R_F units are involved. The program is written in BASIC.

3. PLANAR CHROMATOGRAPHY: SPECIAL TECHNIQUES

3.1. Continuous development

In this mode solvent is allowed to evaporate from the end of the plate and spots continue to migrate until they reach the end of the plate or the plate is removed from the solvent.

Extensive work has been performed by Nurok [39]. The distance migrated by a solute in continuous development TLC is given by a modification of eqn. 1:

$$M_D = \frac{1}{1 + \exp(a \ln X_s + b)} \left[\frac{l^2 - 2lx_0 + \kappa t_l}{2l} \right] \tag{14}$$

where l is the solvent path length, x_0 the spotting distance, κ the solvent velocity constant and t_l the analysis time.

Plots of M_D *versus* X_s permit solvent systems to be compared. To draw the plots l is arbitrarily specified and t is selected such that the least retained solute migrates to the end of the fixed l at the highest mole fraction of X_s. A glance at this plot permits the variation in selectivity to be checked.

3.2. Two-dimensional TLC

In this mode the usual approach is to make use of two developments along two orthogonal directions using two different mechanisms, provided that the sample is spotted in the corner of a square plate. The worst situation is that where all spots lie on a straight line, which means a high degree of correlation. To exploit the capabilities of two-dimensional TLC fully, spots would be spread over the whole plate. Clearly, the larger the spreading the less is the correlation. This is the basis of a very simple strategy proposed by De Spiegeleer *et al.* [40]. Each spot is located and identified by its coordinates x and y and a correlation coefficient R provides a measure of the linearity (or similarity) of the systems. The data matrix contains the hR_F ($R_F \times 100$) values of the k components in the n different chromatographic systems. The correlation matrix of these systems ($n \times n$) is calculated. The lowest absolute value gives the best combination of systems:

$$D\ (k \times n) \rightarrow C\ (n \times n) \rightarrow \min |R| \tag{15}$$

To obtain a percentage value, the correlation criterion is expressed as $100(1 - R)\%$. Silica gel HPTLC plates were used and eleven mobile phases tested. The selection is performed by searching for the lowest absolute correlation coefficient.

Gonnord *et al.* [41] previously used x and y coordinates and proposed two functions:

$$D_A = \sum_{i=1}^{k} \sum_{j=i+1}^{k} [(x_i - x_j)^2 + (y_i - y_j)^2] \tag{16}$$

$$D_B = \sum_{i=1}^{k} \sum_{j=i+1}^{k} \frac{1}{(x_i - x_j)^2 + (y_i - y_j)^2} \tag{17}$$

where D_A sums the square of all possible distances between any pair of spots, the aim being to maximize D_A, and D_B sums the inverse of these distances, the aim being to minimize D_B.

D_B would be undefined for unresolved pairs which are either eliminated or replaced by a distance equal to the average spot width. The response function was tested with nineteen amino acids and the optimization according to D_B yielded a better resolved chromatogram. It must be noted that Bayne and Ma [30] pointed out that one eccentric point may dominate the distance measure. Nurok *et al.* [42] used modifications of D_A and D_B in the two-dimensional TLC separation of steroids. *DF* and *IDF* [43] are of the same form as D_A and D_B but use distances rather than the squares of distances and the *PRF* which is defined by

$$PRF = \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \ln \left(\frac{S_D^{ij}}{S_D^{spec}} \right) \tag{18}$$

where S_D^{ij} is the actual spot separation and S_D^{spec} is the desired spot separation. All solute pairs with $S_D^{ij} > S_D^{spec}$ are assigned a value of S_D^{spec} and have a zero contribution to the *PRF*. The continuous development mode was selected and the different M_D

values were calculated. Plots of computed migration for each steroid *versus* mole fraction of the polar modifier were drawn and visual inspection permitted the scatter of the spots to be checked. According to Nurok [39], *IDF* is less sensitive than *DF* to the presence of poorly resolved pairs.

Visual inspection is difficult to handle since 1681 chromatograms were simulated. To overcome this problem, Steinbrunner *et al.* [44] constructed contour diagrams (Fig. 4). In these diagrams the solvent compositions for the two developments are independent variables and spot separation is the dependent variable. The curves are isoresponse curves for the distance between the most poorly separated pair of solutes. The darkened area indicates the optimum solvent composition. Good agreement was claimed between the simulated and the experimental chromatograms on a dual plate consisting of a strip of C_{18} layer adjacent to silica gel.

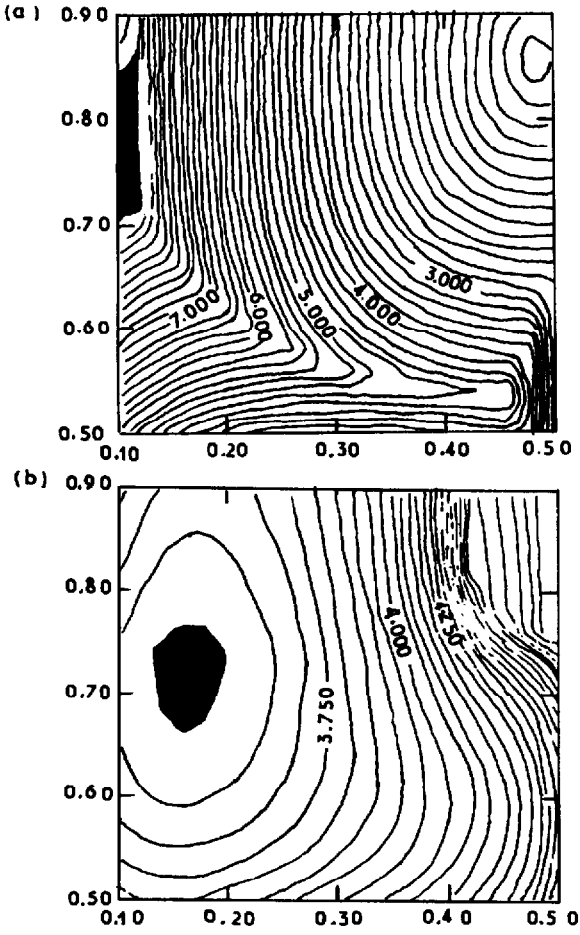

Fig. 4. Two-dimensional contour diagram for the separation of steroids on a dual-phase plate. The shaded area represents the region of the solvent domain where all solutes are separated by an appropriate distance (reprinted from ref. 44, with permission).

However, in our experience discrepancies occur between simulated and experimental runs because a small gap exists between the two layers. In the second development the NP-type solvent is strongly eluting the solutes on the C_{18} layer, which acts as a concentration zone. Solutes are located in the solvent front and spots are elongated perpendicular to the development direction. On reaching the interface between the two layers some mixing occurs, resulting in disturbed zones. It would be very valuable to use a homogeneous plate and polar bonded phases are potentially useful.

4. CONCLUSION

Compared with HPLC, TLC looks simple and rapid. However, in both instances the optimization procedures are time consuming. With the notable exception of the work of Nurok *et al.* [42] little has been done on two-dimensional TLC and continuous development and nothing has appeared on computer-assisted methods for automated multi-development (AMD). Similarly, procedures for the reversed-phase mode are scarce and nothing has appeared on optimization with cyano-bonded phases, which look promising as they can be used in both NP and RP modes [45].

At present silica gel packings are generally considered and the selection of mobile phase is more tedious in TLC than in HPLC owing to the possible solvent demixing and front formation. As it is very difficult to differentiate between spots in the R_F range 0–0.1, the retention of solutes should be limited to the range 0.1–0.8, which means in the ideal case a k' range of 0.25–9. From compilations in the literature, two strategies are utilized: either keeping the solvent simple and considering binary mixtures only, or tuning selectivity by additing more solvents, thus leading to very complex mixtures. In the first instance a limited number of experiments are required (theoretically two) and a window diagram is simple to look for separation and peak cross-overs. A large amount of data is available from the literature but the experimental conditions are not very often clearly indicated. In the second instance the PRISMA model looks powerful.

Some of the criteria proposed for computer-assisted methods in HPLC have been utilized in TLC optimization. In our opinion, resolution-based criteria which make use of spot width are meaningless as the plate count is not constant in TLC. The approach proposed by De Spiegeleer *et al.* [24] looks well suited for TLC.

For the same purpose the *CRF* function should be tried with a procedure other than the simplex method. As visual inspection is still very often used in PC, the analyst has no idea of the peak shape and peak separation is obviously the most useful parameter. When dealing with sample mixtures containing different amounts of solutes, the discrimination factor [46] would be used as a criterion.

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