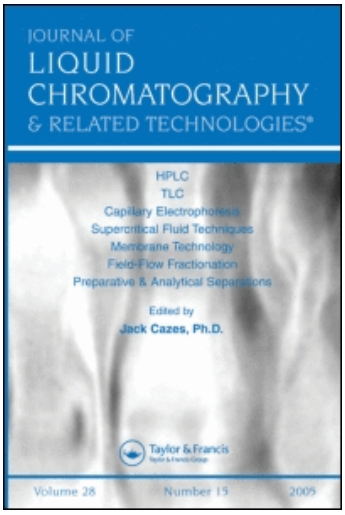

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COMPUTER SIMULATION OF THE SEPARATION IN ONE- AND TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY BY ISOCRATIC AND STEPWISE GRADIENT DEVELOPMENT[1]

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ABSTRACT

A method is presented for the computer-assisted selection of the mode of development for the separation of ten phenolic components. The method is based on simulation of isocratic and gradient development. The different combinations of isocratic and gradient development in one (1D) and two (2D) dimensions are tested. The qualities of chromatograms are evaluated by application of criteria such as the distance function and multipeak criterion.

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INTRODUCTION

Thin-layer chromatography (TLC) has long been considered useful for the rapid and inexpensive qualitative analysis of simple mixtures. During the past 10 years however TLC has undergone a renaissance with the introduction of high performance TLC plates, precise spotting devices, sensitive spectrophotometric scanners (densitometers), full automated development process [1] and it has become accepted as a technique for precise and sensitive qualitative and quantitative analysis [2].

In planar chromatography some form of optimization is generally necessary if complete separation of all components in a sample is required and if the number of components is larger than a small fraction of the spot capacity of the system [3].

In recent years the study of the systematic strategies to optimize systems of HPTLC (TLC) has rapidly gained widespread acceptance combined with computer-assisted method development [4]. The optimization methods are in a very easy way transferred from HPLC to HPTLC, after consideration of differences between them.

The confident analysis from moderate to complex mixtures requires a very large peak capacity that is most readily obtained using multidimensional and multimodal separation techniques. The 2D (two-dimensional) TLC technique offers high capacity [4,5] and it is especially simple, since separations are normally carried out in a plane and

sequential development in orthogonal direction and this is all that is needed.

The advantages of 2D-TLC have been described by Guiochon and Nurok [4-6], who applied this technique to separation of complex mixtures. Their investigations were carried out by isocratic developments. The gradient development, especially the step-gradient TLC has become widely accepted and efficient method for the solution of the so-called general elution problem, i.e., the separation of compounds having greatly differing retentions. Another reasons are the enhancement of mutual displacement of the components to be separated and compression effect of the spots taking place during the gradient development. Considering above statements, it will be very interesting to investigate how the combination of 2D-TLC and the stepwise gradient mode work together and if it is possible to get better resolution of complex mixtures, with wide spectrum of polarity. In an earlier paper [7] an equation for the R_{FG} value of solute chromatographed under stepwise gradient conditions was derived, assuming a definite relationship between the k values and modifier concentration and in the subsequent papers computer programs were elaborated [7-12]. In the present paper equation derived determines the final position of solutes after 2D development, where 1D (one dimensional) developments can be carried out in different modes.

There are several established approaches [13,14] *to optimizing* separation quality using a suitable separation metric.

To judge the quality of an entire chromatogram, elementary criteria, used only for a pair of the peaks must be extended. A first possible combination is the summation of elementary criteria for successive compound pairs. A second class of criteria for the entire chromatogram multiplies the elementary criteria. The advantage of these product criteria is their sensivity, especially for the least resolved compound pairs.

THE THEORETICAL CONSIDERATION

One-dimensional development

The investigated sample is a multicomponent mixture, where components differ strongly in polarity. The development process is run in the ideal condition, in the isocratic as well in the gradient mode. For all components of the mixture a definite relationship between retention and the properties of mobile phase is known. This relationship can be described by the well known equation which follows from the Snyder-Soczewiński adsorption model [16,17] presented in the logarithmic form:

$$\log k_{AB} = \log k_o - m \log C_B \qquad (1)$$

Sometimes the relationship between the retention and the properties of mobile phase can be described by a polynomial form:

$$k_{(l)} = k_{(0)} + k_{(1)} C_{(l)} + k_{(2)} C_{(l)}^2 \qquad (2)$$

The isocratic development

When the chosen development mode is isocratic, then the final R_F values for solutes are determined by the following equation:

$$R_F = \frac{1}{1 + k} \tag{3}$$

The simple stepwise gradient development

If the applied mode is the stepwise gradient mode, then the final R_F values for solutes are determined by two equations:

- for solutes which migrated only in first zone (h = 1) of gradient program (for details see [7,8]) by equation:

$$R_{FG(j)} = \frac{1}{1 + k_{(j,l)}} \tag{4}$$

- for solutes which migrated through more than one zone (h ≥ 2) by equation:

$$R_{FG(j)} = \sum_{l=1}^{h-1} \frac{v_{(l)}}{k_{(j,l)}} + R_{F(j,h)}\left[1 - \sum_{l=1}^{h-1} \frac{v_{(l)}}{1 - R_{F(j,l)}}\right] \tag{5}$$

The unidimensional development

Again if the applied mode is unidimensial development (constant distance and the same mobile phase) then the final R_F value can be determined by equation [18]:

$$R_{FG_{(J)}^{m}} = 1 - (1 - R_{F_{(J)}})^{m} \tag{6}$$

The multiple gradient development

The last mode of development can be multiple development (increased elementary distance of development) where the development distance increase and the concentration of a modifier decreases for the consecutive steps. The details of the derived equations were presented in paper [12]:

$$R_{FG_{(J)}} = S_{(n-1,J)} + [Z_{(n)} - S_{(n-1,J)}] R_{F_{(n,J)}} \tag{7}$$

As mentioned earlier, to judge the quality of the chromatogram in one dimensional development the distance function is used:

$$D^{1D} = \sum_{I=1}^{n-1} \sum_{J=I+1}^{n} [(X_{(I)} - X_{(J)})^2] \tag{8}$$

where $X_{(i)}$, $Y_{(j)}$ are the values of R_F of solutes or distances of migration.

Other function is MPC (multipeak criterium) [15] defined by equation:

$$MPC = \left[R_{F_{(MAX)}} - R_{F_{(n)}}\right]\left[R_{F_{(1)}} - R_{F_{(MIN)}}\right] \prod_{i=1}^{n-1} \frac{R_{F_{(i+1)}} - R_{F_{(i)}}}{\left[\frac{R_{F_{(MAX)}} - R_{F_{(MIN)}}}{n+1}\right]^{n+1}} \quad (9)$$

where:

$R_{F(MAX)}$ $R_{F(MIN)}$ extreme values within which all spots of compounds must lie

$R_{F(1)}$ - corresponds to the component of lowest R_F

$R_{F(n)}$ - corresponds to the component of highest R_F

n - number of components.

Two-dimensional development

The place of spotting the sample is considered as the origin of a coordinate system.

The process of development is carried out in two stages: the first in the direction of the x-axis on the distance L_x and the second (after evaporation of the first solvent) in the direction of y-axis on the distance L_y.

The positions of solutes after development in the x direction depend on the selectivity of the system; mobile phase/adsorbent for isocratic development and additionally on the gradient program if the latter technique is applied. Similarly, the migration distance in the y-direction depends on selectivity, the development distance and the applied gradient program.

After the development in the x-direction, the ordinates of all spots are zero. After the development in the y-direction, their abscissa values follow from their positions on the x axis after the first development.

The final positions of spots are thus determined by the coordinates $X_{(j)}$ and $Y_{(j)}$ which can be calculated from the equation:

$$R_{FXY_{(j)}} = [R_{FX_{(j)}}, R_{FY_{(j)}}] \tag{10}$$

The two-dimensional TLC separation is of no interest if the selection of the two different eluting systems and modes is not adequate. A good separation will be obtained when the surface area of plate over which the spots are spread is relatively large.

It is thus useful to calculate an estimate of the quality of a two-dimensional separation. In our case the distance function will be calculated [6].

In its simplest form the D^{2D} is:

$$D^{2D} = \sum_{l=1}^{n-1} \sum_{j=l+1}^{n} \left[(X_{(l)} - X_{(j)})^2 + (Y_{(l)} - Y_{(j)})^2 \right] \tag{11}$$

where: - $(X_{(i)} - X_{(j)})$ is the distance between an adjacent pair of spots in direction X

- $(Y_{(i)} - Y_{(j)})$ is the distance between an adjacent pair of spots in direction Y.

Equations (1 to 11) form the base to elaborate a computer program that simulates 2D-development in modes I-I, I-G, G-I, G-G, where I - means isocratic development, G - gradient development.

The flow diagram is shown in Fig. 8. The program allows not only for the calculation of the final R_F values of solutes in 1D and 2D development, but also the values of D^{1D}, D^{2D}, MPC for estimation of quality of chromatogram as well as the graphic representation of chromatogram. The program was written in Pascal (6.0).

EXPERIMENTAL

In this paper only the computer simulations were carried out. The group of phenolic compounds investigated by Lodi at all. [19] is a good example sample of which the components span a wide polarity range. From the plots (R_M vs. log φ) presented in paper [19], we estimated values of k_o and m for this group of solutes chromatographed on the diol-layer with the different mobile phases: acetonitrile + dichloromethane (the system I) and acetone + dichloromethane (the system II).

TABLE 1

Phenolic Compounds Used as Standards and Acetonitrile and Acetone as Modifier.

No.	Compound	Acetonitrile + dichloromethane		Acetone + dichloromethane	
		k_o	m	k_o	m
1	Rutin	6.45	3.00	0.330	4.33
2	Kaempferol-3-rutinoside	4.47	3.00	0.240	3.88
3	Quercetin-3-arabinoside	2.69	2.50	0.204	3.59
4	Quercetin-3-galactoside	1.91	2.85	0.120	3.30
5	Chlorogenic acid	1.02	1.80	0.072	2.88
6	Myricetin	0.99	1.38	0.051	2.49
7	Caffeic acid	0.50	1.30	0.056	1.77
8	Quercetin	0.50	1.03	0.054	1.59
9	Apigenin	0.27	0.97	0.052	1.42
10	Ferulic acid	0.24	0.45	0.035	1.30

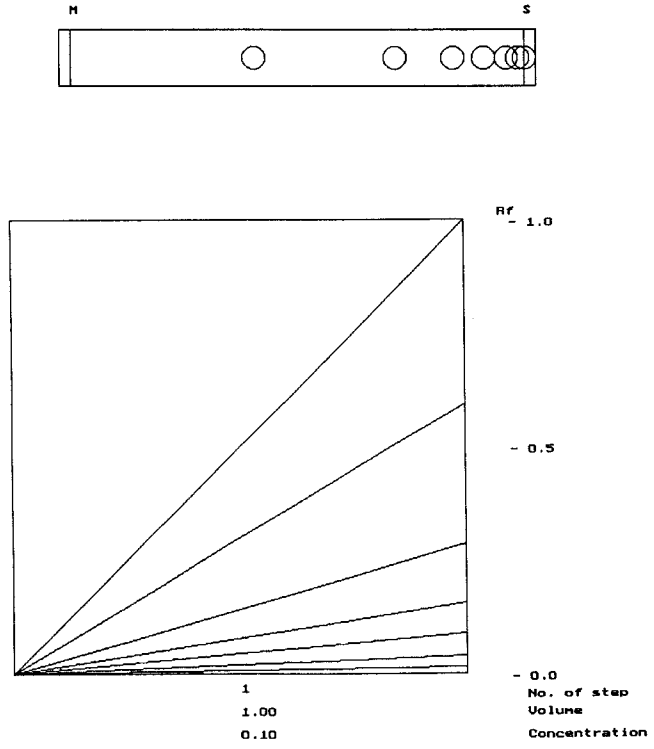

Fig.1. Computer simulated migration paths and chromatogram of ten phenolic components in system I obtained by isocratic development.

In both systems good selectivity is noticed relative to all pairs solutes.

Let us consider the isocratic mode of development for the system I. The optimal range of k values for most analysis by TLC is from k = 0.75 to k = 10 (this corresponds to R_F = 0.57 and R_F = 0.09). The solute of code 1 is the most strongly retained component of mixture. The

lowest concentration of modifier giving optimal range of k for the most strongly retained component is $\Phi = 0.46$ ($R_F = 0.10$). At this concentration the solutes of codes 5 to 10 are over the optimal range (Table 2). For the concentration of modifier higher than $\Phi = 0.46$ the components of codes 7, 8, 9, 10 have values k lower than 0.75 and will be accumulated up at the front of the mobile phase. When the concentration of modifier is lower than 0.46, the successive components of mixture have values of k higher than 10. From the above analysis it results that the isocratic mode of development is not satisfactory for the investigated components. Similar conclusion can be drawn from the analysis for system II. Table 2 presents the R_F values calculated for the minimal concentration of modifier, which gives optimal range for the most strongly retained components in system I and II. The quality of such chromatograms from the point of optimal range k values is not good considering all components.

The application of the stepwise gradient should improve the distribution of solutes on chromatogram.

For gradient application the main parameters having influence on the final values of R_F are: the initial concentration of modifier, the way of changing the concentration, the number of the steps and the volume of steps. The computer program which is able to predict the final values of R_F is very useful to simulate the gradients with many different values

TABLE 2

The R_F Values (>0.05) of Investigated Solutes for the Minimal Concentration of Modifier.

Code	System I	System II
	c_{mod} = 0.85	c_{mod} = 0.46
1	0.04	0.10
2	0.06	0.17
3	0.12	0.23
4	0.14	0.39
5	0.32	0.60
6	0.36	0.74
7	0.54	0.82
8	0.57	0.84
9	0.71	0.86
10	0.78	0.91

of parameters. The results of simulations of different gradients applied to system I and II are presented in Figures 2 and 3 and in Table 3.

At first, the simplest two-step gradient was considered and the system I was chosen. It was assumed that least retained solute should

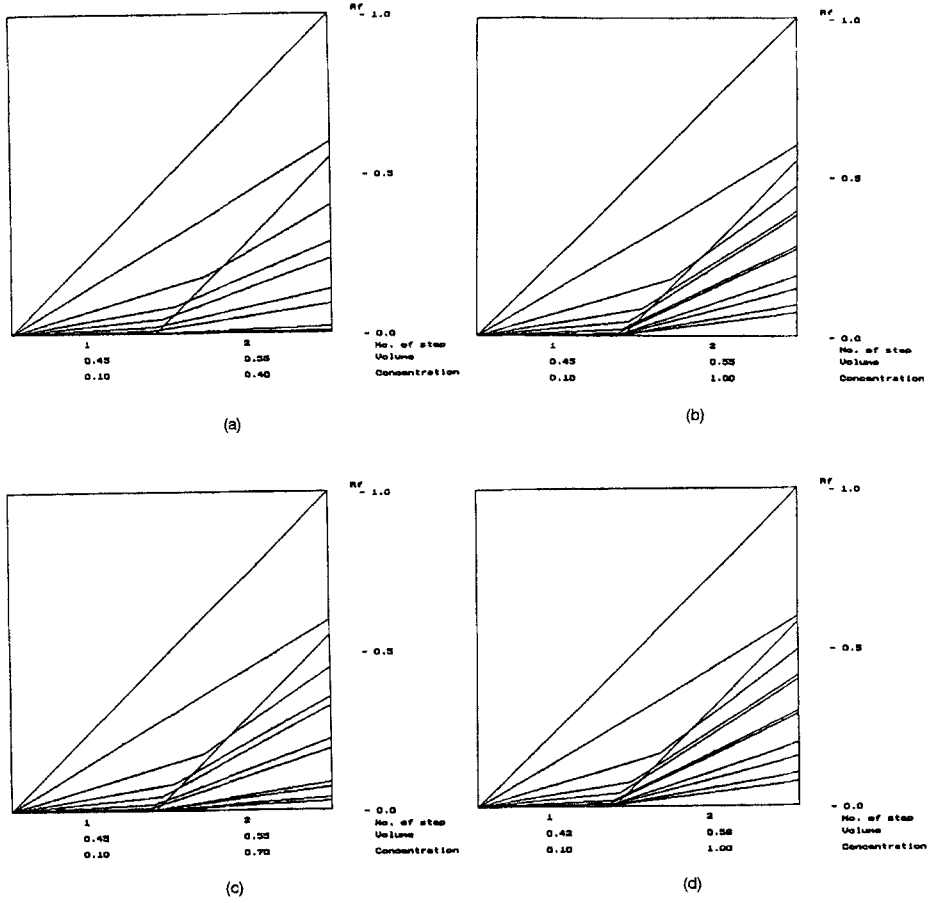

Fig.2. Computer simulated migration paths of ten phenolic components for different programs of gradient in system I.

have the R_F value equal to 0.6. The initial concentration of modifier can be calculated by equation (1) with the known values of k_o and m. It was $\Phi = 0.4$ for system I. The simulation of the isocratic development with concentration 0.4 of modifier shows separation of solutes. Only the

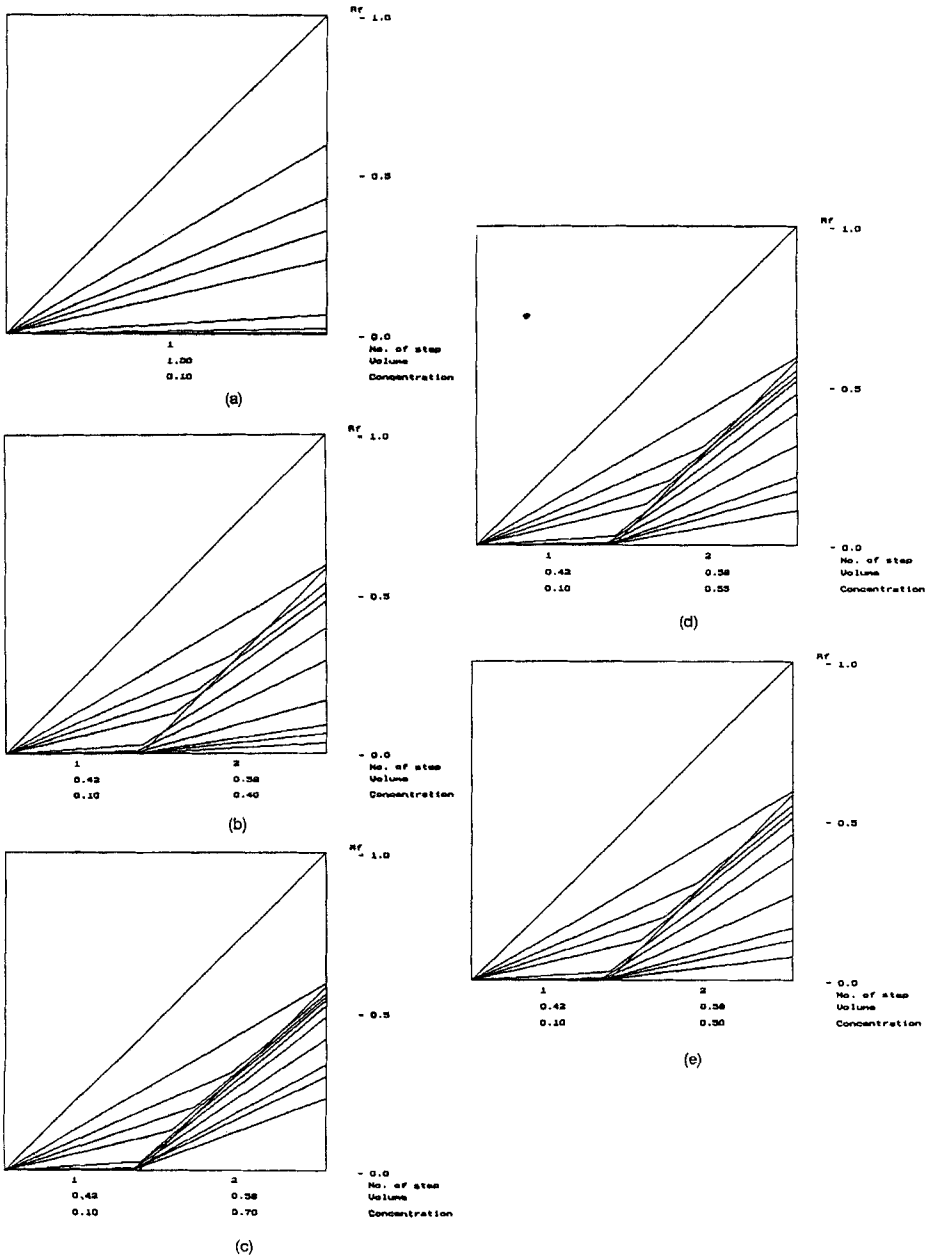

Fig.3. Computer simulated migration paths of ten phenolic components for isocratic and gradient developments in system II.

TABLE 3

The Characteristic of Simulated Separation in 1D Mode Obtained by the Isocratic and the Gradient Development.

No.	Figure	The range of R_F	Mode	Quality of chromatograms	
				D^{1D}_A	MPC [%]
1.	1	0.00 ÷ 0.60	ISO	3.3249	0.00
2.	2a	0.01 ÷ 0.60	GRAD	3.5916	0.00
	2b	0.03 ÷ 0.60	GRAD	3.3221	0.93
	2c	0.07 ÷ 0.60	GRAD	2.6076	3.97
	2d	0.08 ÷ 0.60	GRAD	2.6224	4.48
3.	3a	0.00 ÷ 0.59	ISO	4.1484	0.00
	3b	0.03 ÷ 0.59	GRAD	4.1724	8.07
	3c	0.23 ÷ 0.59	GRAD	1.4176	1.35
	3d	0.11 ÷ 0.59	GRAD	2.7669	10.76
	3e	0.08 ÷ 0.59	GRAD	3.2581	19.93

solutes of codes 7 to 10 have R_F values from optimal range and between solutes of codes 9 and 10 there is large gap (0.3 R_F unit).

From Fig. 2a, it follows that only solute of code 10 should be eluted in the first step. The volume of this step is 0.45. The next step is to choose the concentration of modifier for the next step and check if it is adequate and if all solutes are in the optimal range. If this condition is

fullfilled then the program is satisfactory. Next simulations can only refine the program. In other case, when two-step gradient is insufficient, the procedure is repeated until the suitable program is found.

Fig. 2a, b, c shows successive steps in choosing the gradient program. Fig. 2d presents refined separation. Similar simulation was done for the system II. The results are presented in Fig. 3a-e. In this system, the concentration of modifier in the second step $\Phi = 0.7$ was too high and in the next simulation, the values from the range $0.4 \div 0.7$ was chosen. It follows from above simulations that two-step gradient efficiently separates ten solutes in the optimal range of k.

The chromatograms obtained can be evaluated visually and with suitable quantitative criteria. In our case three criteria were used: optimal range of R_F values, the D criterion (the higher D value the better the separation) and the MPC criterion proposed by De Spiegeleer [15]. The results are presented in Table 3. The fulfillment of all three criteria in a satisfactory degree indicates a good result. In the case of system I the results represented in Fig. 2d may be considered satisfactory in spite of low value of MPC which is explained by low selectivity of the system relative to some pairs of components. It follows from Figs. 3 a-e and Table 3 that the use of system II for the separation of the investigated ten - component mixture gives considerably better results. The chromatograms obtained are characterized by considerably better values of criteria, both D as well as MPC. The chromatogram shown in Fig. 3e secures optimal

range of R_F values, moderately high D index and the highest MPC index which characterizes the regular distribution of spots in the required range of R_F values.

What are the advantages of combining isocratic and gradient development in the two - dimensional (2D)technique? Gradient elution permits the choice of such a programme that the investigated solutes of wide range of polarities are distributed along the whole distance of development. If the system chosen is highly selective relative to each pair of components, then in the second (orthogonal) distance a isocratic eluent selective to the most difficult pair (or several pairs) of components can be chosen. Another solution consists in consecutive steps corresponding to most selective systems for the individual difficult pairs. The simulations of 2D developments are presented in Fig.4-7 and Table 4. The latter lists the values of criterion of evaluation of chromatogram calculated according to eq.11 assuming equal development distances (10 cm) in direction X and Y.

It is known that the use of the same system for 2D development results in insignificant improvement of separation efficiency; $R_S^{2D} \approx R_S^{1D} \sqrt{2}$ which is illustrated in Fig. 4; for instance, for solutes 1 and 2 the separation distance is 2 mm after 1D and and 2.83 mm after 2D development. A similar insignificant increase of distance is observed for solutes 7 and 8. Since selectivity was the same for both systems, the spots are located after 2D development on a diagonal line.

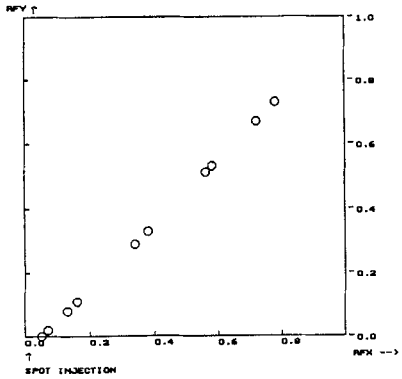

Fig. 4.

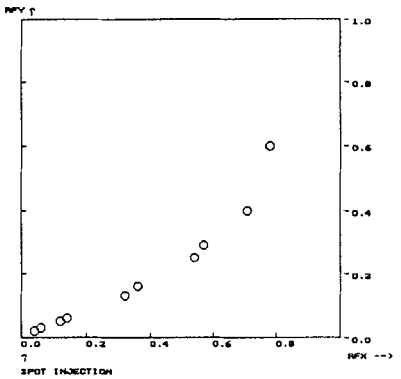

Fig. 5.

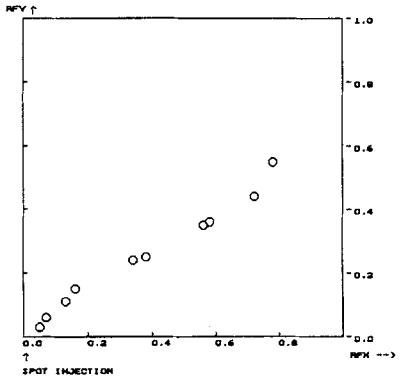

Fig. 6.

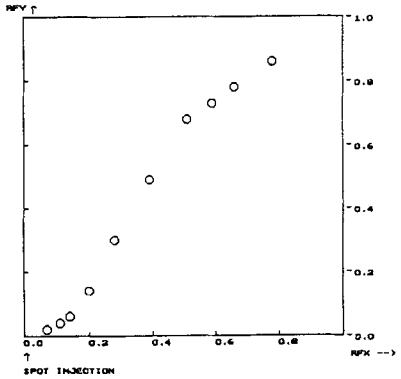

Fig. 7.

Fig.4 to Fig.7. Computer simulated chromatograms for different combinations of development mode.

Fig. 5 illustrates the use of combined isocratic and gradient development. Isocratic elution distributes the components along the whole distance of development; the shortest distances are observed for solutes 1, 2 (2 mm) and 7, 8 (2 mm). The use of gradient results in increased distance between solutes 1 and 2 (by 3 mm) but only by 1 mm

TABLE 4

The Characteristic of Simulated Separation in 2D Mode Obtained by Combination the Isocratic and Gradient Development.

No.	Figure	Mode 2D	D^{2D}	System
1.	4	ISO - ISO	133 082	I
2.	5	ISO - GRAD	92 765	I
3.	6	ISO - GRAD	99 573	I
4.	7	GRAD - ISO	158 561	II

between solutes 7 and 8. The final distances between solutes 1 and 2 are 3.61 mm and between 7 and 8 2.36 mm. On the other hand, distances between the remaining components are markedly increased.

Fig. 6 illustrates the case when the pair 7 + 8 is not separated but the components are eluted in the suitable range of R_F values. The choice of gradient programme in which the proper pair 7, 8 will be separated improved the overall separation too; the final distance between 7 and 8 is increased from 0 to 2 mm.

Further simulations in the system Iso-Grad permitted to get a still better final separation. Fig. 7 illustrates the result of Iso-Grad 2D development in which the distance between solutes 7 and 8 is increased to 5 mm.

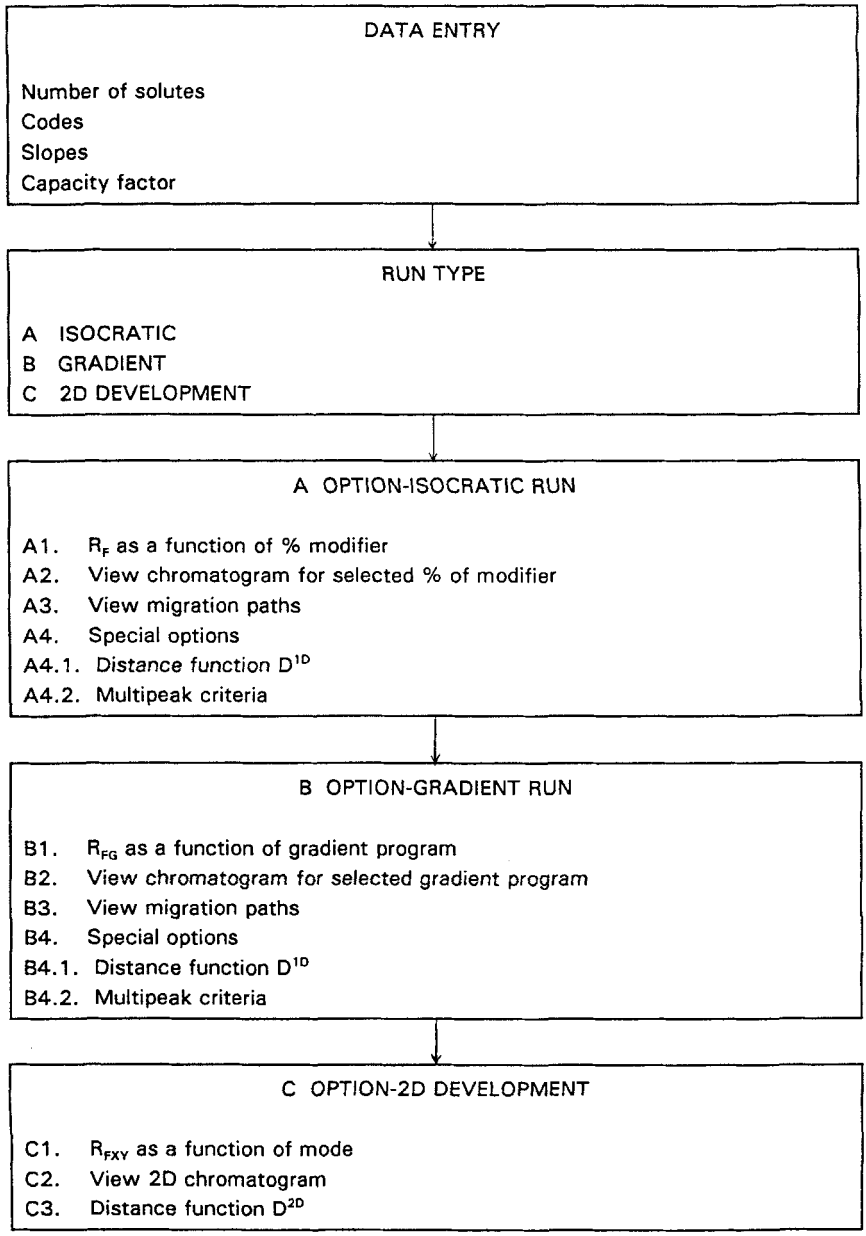

Fig.8. Flow diagram of computer program used to simulation of isocratic and gradient development in 1D and 2D.

Similar simulations were applied to system II where for 1D gradient elution was used, followed by isocratic elution for the second dimension. All pairs are well separated which is confirmed by the high value of D^{2D} = 158 000.

The programme elaborated for the isocratic and the gradient development in combination permits:

- the simulation of 2D development
- obtaining the diagram of 2D chromatograms
- calculation of values used for the estimation of chromatograms

The application of 2D simulation can be especially useful in the case of multicomponent mixtures of wide range of polarity to utilize the high capacity offered by 2D-TLC. It follows also from the above simulations that the investigated mixture of ten phenolics can be separated using a simple stepwise gradient or its combination with isocratic elution without necessity of use of time - consuming multiple development.

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