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COMPUTER-ASSISTED SELECTION OF THE OPTIMUM GRADIENT PROGRAMME IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A general equation for the final R_F value of a solute chromatographed under conditions of stepwise gradient development with one void volume of mobile phase has been formulated. On the basis of this equation a computer program (in BASIC) is given for the automated selection of the optimum gradient programme. The final values of *Rfg* and *Rsg* are calculated and compared with experimental data. Comparison of the results showed satisfactory agreement.

INTRODUCTION

Gradient development has become a widely accepted and efficient method for the solution of the so-called general elution problem[1–3], *i.e.*, the separation of compounds having greatly differing retentions. Another reason is the enhancement of mutual displacement of the components to be separated. A simple version of the stepwise gradient development in thin-layer chromatography (TLC) is possible for equilibrium sandwich chambers with a glass distributor[4–7] or the technique called the programmed multiple developing process[8–10].

In an earlier paper[11], an equation for the *Rfg* value of a solute chromatographed under stepwise gradient conditions was derived, assuming a definite relationship between the k' value and modifier concentration. In subsequent papers[12,13], a microcomputer program was presented that simplifies the calculation of the final *Rfg* values obtained for stepwise gradient development. The gradient program (number of steps, concentrations of modifier and volumes of individual steps) was selected by the trial-and-error method.

This paper deals with the automated selection of the optimum gradient programme, *i.e.*, the selection of the three parameters that characterize the programme. The selection is made by computer and is based on previously determined assumptions. The quality of the chromatogram is estimated from the relative resolution introduced by Drouven[14] and adapted for TLC. The program (see Appendix) was written in BASIC (Microsoft version 2.04).

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THEORETICAL

The process of gradient development in TLC will be considered, *e.g.*, using a sandwich chamber (TLC-S)[4–7]. A binary eluent is employed; the sum of the eluent volumes introduced into the layer in the individual steps is equal to the void volume (V_m) of the layer.

The retention–solvent composition relationship of the solutes for given adsorbent–eluent systems is assumed to be described by one of the well known equations:

$$\text{normal phases: } k(j,i) = \frac{k_0(j)}{c(i)^{m(j)}} \tag{1}$$

$$\text{reversed phases: } k(j,i) = \frac{k_0(j)}{10^{m(j)c(i)}} \tag{2}$$

or, for more complex cases:

$$k(j,i) = A0(j) + A1(j) \log c(i) + A2(j)[\log c(i)]^2 \tag{3}$$

where

$k(j,i)$ = capacity factor of solute j for the ith consecutive gradient step;

$k_0(j)$ = capacity factor of solute j for unit concentration of modifier (volume fraction, $c = 1$);

$m(j)$ = slope of the log–log plot for solute j;

$A0(j)$, $A1(J)$, $A2(J)$ = constants characteristic of solute j.

The gradient programme should be chosen so that all solute pairs are separated and the spots are uniformly spread along the chromatogram. To characterize the separation of solute pairs, the criterion Rsg will be used (resolution in gradient development); to estimate the uniformity of spread of spots, the criterion of relative separation, r^*, will be defined.

The resolution is defined as

$$Rsg(k/j) = \frac{2[Rfg(k) - Rfg(j)]}{w(k) + w(j)} \tag{4}$$

where

$Rfg(j)$ = final R_F value of solute j;

$Rfg(k)$ = final R_F value of solute k;

$Rsg(k/j)$ = resolution of solutes k and j.

Assuming that the final spreading of the solute spots (along the direction of development) is equal to 0.05 R_F units, we can characterize the separation by the approximate equation

$$Rsg \approx 20 \Delta Rfg \tag{5}$$

where $w(k) \approx w(j) \approx 0.05$.

The relative criterion of separation is calculated from the equations

$$h(j) = Rfg(k) - Rfg(j) \tag{6}$$

$$r^* = \frac{\prod_{j=1}^{b-1} h(j)}{\left[\frac{\sum_{j=1}^{b-1} w(j)}{b-1}\right]^{b-1}} \tag{7}$$

The meaning of *Rsg* is generally known; the relative separation r^* characterizes the uniformity of spreading of spots along the chromatogram ($0 \leqslant r^* \leqslant 1$).

The algoritm elaborated in this study consists of the following steps. In the first step, the minimum distance between the spots is defined. If we assume that $Rsg = 1$, then it corresponds to the difference $\Delta Rfg \approx 0.05$; for $Rsg = 1.5$ the difference is equal to 0.075. In the second step, the maximum distance between the spots is chosen; it can be calculated as ratio of the development distance by the number of solutes in the mixture; $h\max = 0.95/b$. The third step defines the R_F value of the least retained solute (highest R_F value). It is calculated as the product of the number of solutes in the mixture and the minimum distance between solute spots, or, $Rfg1 = bh\min$.

The criterion being thus defined, we can proceed to choose the gradient programme. We assume a model b-component mixture; the $k_0(j)$ and $m(j)$ values are known for all components. During consecutive steps, eluent fractions of composition $c(i)$ (volume fraction units) and volume $v(i)$ are introduced into the layer. In the calculations, equations derived in a previous paper[11] are used; these describe the migration of solutes in the consecutive concentration zones and permit the calculation of the final *Rfg* values.

The path migrated by solute j in the ith step [volume $v(i)$, concentration $c(i)$] is given by

$$y(j,i) = v(i) \cdot \frac{Rf(j,i)}{1 - Rf(j,i)} \tag{8}$$

The retention volume corresponding to the migration of solute on the distance of $y(j,i)$ is given by

$$x(j,i) = \frac{v(i)}{1 - Rf(j,i)} \tag{9}$$

where $Rf(j,i)$ is the R_F value of solute j for the ith step and $v(i)$ is the volume of eluent for the ith step.

The final *Rfg* values can be calculated from eqns. 10 and 11, depending on the number of concentration zones through which the solute band migrated (Fig. 1):

$$Rfg(A) = V_m \cdot Rf(A,1) \tag{10}$$

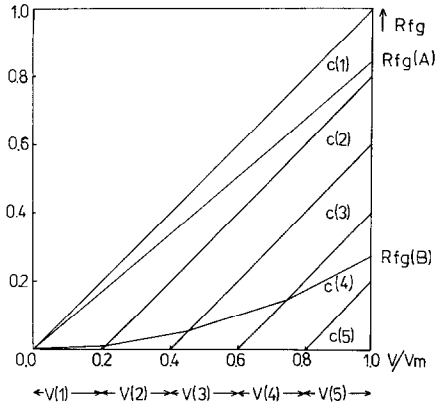

Fig. 1. Migration of solutes A and B under conditions of a five-step gradient.

$$Rfg(B) = \sum_{i=1}^{h-1} y(B,i) + Rf(B,h)\left[1 - \sum_{i=1}^{h-1} x(B,i)\right] \tag{11}$$

$$Rfg(B) = \sum_{i=1}^{h-1} v(i) \cdot \frac{Rf(B,i)}{1 - Rf(B,i)} + Rf(B,h)\left[1 - \sum_{i=1}^{h-1} \frac{v(i)}{1 - Rf(B,i)}\right] \tag{12}$$

Eqn. 10 corresponds to the final R_F value in the case when the solute band is eluted solely in the first concentration zone; eqns. 11 and 12 correspond to the migration of solute through h ($h \geqslant 2$) concentration zones.

The first stage of optimization of the gradient programme consists in the choice of such a concentration of the modifier that the first solute has $Rfg = Rf1$. For this purpose, eqn. 1 is transformed to calculate the concentration for which this condition is fulfilled. The solutes are denoted by numbers 1–b, attributing to their definite codes

$$c(j) = \left[\frac{k_0(j)}{k(j,1)}\right]^{\frac{1}{m(j)}} \tag{13}$$

The calculations result in a series of concentrations $c(j)$, from which the lowest is chosen; the choice of low modifier concentration is favourable for a high selectivity of the system.

The next step consists in the calculation of R_F values for all solutes as if the development was isocratic, the concentration of modifier corresponded to $c$min and the volume of first step corresponded to the void volume V_m of the system. The R_F values are calculated from the equations

$$k(j,i) = \frac{k_0(j)}{c\mathrm{min}^{m(j)}} \tag{14}$$

$$Rf(j,i) = \frac{1}{1 + k(j,i)} \tag{15}$$

The R_F values are then ordered from the smallest to the greatest; the solute with the lowest value is denoted No. 1 and that with the greatest value No. b. The ordering of R_F values is accompanied by ordering the codes of the solutes as well as slopes $m(j)$ and values of $k_0(j)$. The following series is obtained:

$$Rf(1,1), < \ldots < Rf(j-1,1) < Rf(j,1) < Rf(j+1,1) < \ldots < Rf(b,1) \tag{16}$$

For consecutive pairs of solutes the differences in R_F values are calculated and it is checked whether the difference fulfills the following condition:

$$h = Rf(j,1) - Rf(j-1,1);\ h \geqslant h\text{min and } h \leqslant h\text{max} \tag{17}$$

When this condition is fulfilled for a given pair of solutes, we pass to the next pair. If for any pair of solutes $h = 0$, it means that the pair is eluted in the same step (concentration zone) and the consecutive solute pair is considered. When the condition is fulfilled for all pairs, this corresponds to isocratic development and there is no need to apply gradient development.

Let us assume that for solutes l and $l-1$ the above condition is not fulfilled. Then it is necessary to apply the second gradient step, which would accelerate the migration of solutes $l-1$ to 1; solute l is considered to be eluted last in the first concentration zone.

Now it is necessary to calculate the final Rfg values obtained for solutes eluted in the first concentration zone and for the remaining solutes which are overtaken by the second concentration zone, the latter being necessary for further calculations. The final Rfg values for solutes eluted in the first zone, denoted by $j=b$ to $j=l$, are

$$Rfg(j) = Rf(j,1) \tag{18}$$

The volume of eluent for the first stage is

$$v(1) = 1 - Rfg(l) \tag{19}$$

The volume $v(1)$ is the volume of the first step for which the eluent concentration is $c(1) = c\text{min}$. We introduce another indicator $p(i)$, where i denotes the number of the solute eluted last in the given step 1.

For the remaining solutes which cannot be eluted in the first zone, it is necessary to calculate the following values which are needed for the calculation of final Rfg values, assuming that two-step gradient would be sufficient:

$$sv(1) = v(1) \tag{20}$$

$$sx(j) = x(j,1) \text{ for } j = p(1) - 1 \text{ to } j = 1 \tag{21}$$

$$sy(j) = y(j,1) \text{ for } j = p(1) - 1 \text{ to } j = 1 \tag{22}$$

$$sz(j) = z(j,1) \text{ for } j = p(1) - 1 \text{ to } j = 1 \tag{23}$$

We now assume that the volume of the second step (eluent fraction) is equal to the difference between the void volume and the volume of the first step; the concentration of the second step should be chosen to fulfil the conditions discussed in the Introduction. As we know the *Rfg* value of the solute eluted last in the first step, *Rfg*[*p*(1)], we can now calculate the *Rfg* value of the consecutive solute eluted in the second zone:

$$g1 = Rfg[p(1)] - 0.5(h\text{min} + h\text{max}) \tag{24}$$

$$g2 = sz[p(1) - 1] \tag{25}$$

where $g1$ denotes the *Rfg* value for solute $j = p(1) - 1$, assuming a two-step gradient, and $g2$ denotes the distance migrated by the solute in the first concentration zone. Three cases should be considered. The first occurs when $g1 > g2$; then the solute should migrate in the second concentration zone a certain distance to fulfil the condition of separation, and from this distance we can calculate the corresponding necessary concentration. The cases corresponding to $g1 = g2$ and $g1 < g2$ are physically unreal because they would mean that the solute spots migrate backwards in the second step. To simplify the situation, we assume that the solute should migrate at least a small distance g. The distance is $g = g3 \cdot 0.01$, where $g3$ denotes the difference between *Rfg*[*p*(1)] and *sz*[*p*(1) − 1].

To recapitulate,

$$\text{if } g1 > g2 \text{ then } g = g1 - g2 \tag{26}$$

$$g1 < g2 \text{ then } g = g3 \cdot 0.01 \tag{27}$$

To calculate the concentration for the second stage, the following equations are used:

$$Rf[p(1) - 1,2] = \frac{g}{1 - sx[p(1) - 1]} \tag{28}$$

$$k[p(1) - 1,2] = \frac{1 - Rf[p(1) - 1,2]}{Rf[p(1) - 1,2]} \tag{29}$$

$$c(2) = \frac{k_0[p(1) - 1]^{\frac{1}{m[p(1)-1]}}}{k[p(1) - 1,2]} \tag{30}$$

From the known concentration in the second step, the parameters for all solutes [from $j = p(1) - 1$ to $j = 1$] are calculated:

$$k(j,2) = \frac{k_0(j)}{c(2)^{m(j)}} \tag{31}$$

$$Rf(j,2) = \frac{1}{1 + k(j,2)} \tag{32}$$

$$y(j,2) = Rf(j,2)[1 - \mathrm{sx}(j)] \tag{33}$$

$$sy(j) = sx(j) + y(j,2) \tag{34}$$

The sum of distances thus calculated corresponds to the situation of a two-step gradient; the $sy(j)$ values denote then the final Rfg values after two steps:

$$Rfg(j) = sy(j) \tag{35}$$

As before, the $Rfg(j)$ values are ordered from the smallest to the largest:

$$Rfg(1) < \ldots < Rfg(j) < Rfg(j+1) < \ldots < Rfg[p(1) - 1] \tag{36}$$

For the subsequent pair of solutes which do not fulfil the condition $h \geqslant h\mathrm{min}$ and $h \leqslant h\mathrm{max}$, the indicators l and $l - 1$ are obtained. The last solute eluted in the second zone is denoted as $p(2) = l$. The volume of the second step is then calculated as in the case of the first step:

$$v(2) = 1 - v(1) - Rfg[p(2)] \tag{37}$$

For solutes which continue to migrate after two steps, it is necessary to calculate the distance migrated in the second zone and the total distance migrated in the two zones, and the sum of volumes for two steps:

$$sv(2) = \sum_{i=1}^{2} v(i) \tag{38}$$

$$z(j,2) = v(2) \cdot \frac{Rf(j,2)}{1 - Rf(j,2)} \tag{39}$$

$$sz(j) = \sum_{i=1}^{2} z(j,i) \tag{40}$$

Then it is necessary to calculate the volume corresponding to migration in the second step and the sum of volumes for the two steps:

$$x(j,2) = v(2) \cdot \frac{1}{1 - Rf(j,2)} \tag{41}$$

$$sx(j) = \sum_{i=1}^{2} x(j,i) \tag{42}$$

If the indicator $p(2) = 1$, it means that all solutes are eluted in the second step, *i.e.*, a two-step gradient is sufficient. On the other hand, when $p(2) > 2$, the procedure discussed for step 2 is repeated. Before the continuation of calculations for the third

step, the solutes should be arranged again in the sequence of increasing $sz(j)$ values; analogous ordering should be applied to $k_0(j)$ and $m(j)$ values.

Analysis of the equations for the first two steps shows that they can be generalized, except for the first step. Let us assume that an h-step gradient is to be carried out. The index of the solute eluted last in the $(h-1)$th gradient step is $p(h-1)$. Let us calculate the Rfg value of solute $j = p(h-1) - 1$ from the equations

$$g1 = Rfg[p(h-1)] - 0.5(h\text{min} + h\text{max}] \tag{43}$$

and

$$g2 = sz[p(h-1) - 1] \tag{44}$$

where $g1$ denotes the Rfg value of solute $j = p(h-1) - 1$, assuming an h-step gradient, and $g2$ denotes the distance migrated by this solute after $h - 1$ steps; $g3$ denotes the difference:

$$g3 = Rfg[p(h-1)] - sz[p(h-1) - 1] \tag{45}$$

If $g1 > g2$ then $g = g1 - g2$; when $g1 < g2$ then $g = 0.01 \cdot g3$ and the Rf value is calculated from the equation

$$Rf[p(h-1) - 1,h] = \frac{g}{1 - sx[p(h-1) - 1]} \tag{46}$$

and the remaining parameters from

$$k[p(h-1) - 1,h] = \frac{1 - Rf[p(h-1) - 1,h]}{Rf[p(h-1) - 1,h]} \tag{47}$$

The known value of k' permits the calculation of the concentration required for the hth step:

$$c(h) = \left\{\frac{k_0[p(h-1) - 1]}{k[p(h-1) - 1,h]}\right\}^{\frac{1}{m[p(h-1)-1]}} \tag{48}$$

For solutes $j = p(h-1) - 1$ to $j = 1$ the remaining parameters are calculated:

$$k(j,h) = \frac{k_0(j)}{c(h)^{m(j)}}; \qquad Rf(j,h) = \frac{1}{1 + k(j,h)} \tag{49}$$

$$y(j,h) = Rf(j,h)[1 - sx(j)] \tag{50}$$

$$sy(j) = sz(j) + y(j,h) \tag{51}$$

Next the $sy(j)$ values are ordered from the smallest to the largest, the largest value corresponding to the index $j = p(h - 1) - 1$:

$$sy(1) < \ldots < sy(j-1) < sy(j) < sy(j+1) < \ldots < sy[p(h-1) - 1] \tag{52}$$

Then we check if for the consecutive pairs the following condition is fulfilled:

$$h = sy(j) - sy(j-1); \qquad h \geqslant h\text{min and } h \leqslant h\text{max}$$

If this condition is fulfilled for all pairs of solutes, the $p(h) = 1$, which means that the gradient is terminated in the hth step. We can then write that for solutes with indices $j = p(h - 1) - 1$ to $j = 1$,

$$Rfg(j) = sy(j) \tag{53}$$

and the volume of the hth step is

$$v(h) = 1 - \sum_{i=1}^{h-1} v(i) \tag{54}$$

On the other hand, when condition 50 is not fulfilled for a pair of solutes of indices l and $l - 1$, then the solute l is the last one eluted in the hth step; the calculations are continued for step $h + 1$. The gradient programme is terminated when the index $p(h + 1) = 1$, *i.e.*, when all solutes in the mixture are eluted. The maximum number of gradient steps, $(h + 1) \leqslant b$, corresponds to the number of components of the mixture.

In this way we obtain the characteristics of the programme from the viewpoint of the number of steps, their volumes and the concentrations of the modifier in the consecutive steps. The information is obtained in numerical form; moreover, the *Rsg* values are calculated for each solute pair, in addition to the relative resolution r^* which characterizes the distribution of the solute spots along the chromatogram.

The purpose of this paper is to illustrate the above method of determination of the optimum gradient programme and its computer simulation for various starting conditions. Another aim is to compare the theoretically predicted final *Rfg* values with experimental values.

EXPERIMENTAL

A mixture of five coloured components was chromatographed to a distance of 15 cm in equilibrium sandwich chambers[15] produced by Zakład Odczynników Chemicznych (Lublin, Poland). Precoated silica gel Si 60 HPTLC plates (E. Merck, Darmstadt, F.R.G.) were used. In the stepwise gradient development experiment, the fractions of the eluent corresponding to the elaborated program (Table II) were introduced directly under the distributor. The samples were spotted through a narrow slit in the glass cover-plate after two void volumes of the first eluent fraction had passed through the thin layer of adsorbent. The solvent flow was observed by means of a non-retained azulene marker. The migration of the dyes was recorded visually. Calculations were performed on an IBM XT computer.

TABLE I

THEORETICALLY CALCULATED GRADIENT PROGRAMMES FOR NINE COMPONENTS, FINAL VALUES OF *Rfg*, RESOLUTION OF PARTICULAR PAIRS OF SOLUTES AND RELATIVE RESOLUTION PRODUCT

Minimum difference in R_F values, *h*min = 0.05; maximum difference in R_F values, *h*max = 0.1.

R_F value of first solute	*Relative resolution, r**	*Step*	*Volume*	*Concentration*	*Code*	*Rfg*	*Rsg[j/(j − 1)]*	
Rf1 = 0.5	0.5195	1	0.05	0.042	*s*1	0.94	1.50	s1/s2
		2	0.075	0.057	*s*2	0.87	1.50	s2/s3
		3	0.075	0.109	*s*3	0.79	1.50	s3/s4
		4	0.075	0.208	*s*4	0.72	1.50	s4/s5
		5	0.075	0.394	*s*5	0.64	1.50	s5/s6
		6	0.75	0.74	*s*6	0.57	1.50	s6/s7
		7	0.574	1.00	*s*7	0.45	4.16	s7/s8
					*s*8	0.24	3.15	s8/s9
					*s*9	0.08		
Rf1 = 0.75	0.8312	1	0.25	0.017	*s*1	0.74	1.50	s1/s2
		2	0.074	0.05	*s*2	0.67	1.50	s2/s3
		3	0.075	0.094	*s*3	0.59	1.50	s3/s4
		4	0.075	0.176	*s*4	0.52	1.50	s4/s5
		5	0.075	0.325	*s*5	0.44	1.50	s5/s6
		6	0.075	0.59	*s*6	0.37	1.50	s6/s7
		7	0.374	1.00	*s*7	0.29	2.72	s7/s8
					*s*8	0.15	2.05	s8/s9
					*s*9	0.05		
Rf1 = 0.5	0.2659	1	0.504	0.009	*s*1	0.49	1.50	s1/s2
		2	0.075	0.039	*s*2	0.42	1.50	s2/s3
		3	0.075	0.07	*s*3	0.34	1.50	s3/s4
		4	0.075	0.123	*s*4	0.27	1.50	s4/s5
		5	0.075	0.206	*s*5	0.19	1.50	s5/s6
		6	0.147	0.308	*s*6	0.12	1.46	s6/s7
					*s*7	0.04	0.70	s7/s8
		7	0.035	0.126	*s*8	0.01	0.18	s8/s9
		8	0.012	0.252	*s*9	0.00		

TABLE II

ABSOLUTE SLOPES *m*(*j*) AND VALUES OF CAPACITY FACTORS $k_0(j)$ OF FIVE TEST SOLUTES USED IN THE SELECTION OF THE OPTIMUM GRADIENT PROGRAMME

Code	*Compound*	*Slope, m(j)*	*Capacity factor, $k_0(j)$*
B	Sudan IV	1.72	0.016
D	Sudan III	1.66	0.028
E	*p*-Hydroxyazobenzene	1.72	0.063
H	*p*-Nitroaniline	2.08	0.100
K	Methyl Red	3.12	0.158

RESULTS AND DISCUSSION

Let us consider first a model mixture of nine components. It is assumed that the slope of the $\log k'$ *vs.* $\log c$ relationship is constant for all components and equal to $m = 2$; the capacity factors of all components form a geometrical series and are related to the modifier concentration by the equation

$$k_0(j) = \frac{25.6}{2^j} \tag{55}$$

where $j = 2, 4, 6, \ldots, 18$.

$$\log k(j,i) = \log k_0(j) - m(j) \cdot \log c(i) \tag{56}$$

where $k_0(j) = k(j,i)$ for $c(i) = 1$.

The procedure described above resulted in the programme presented in Table I, which was calculated assuming that $h\text{min} = 0.05$, $h\text{max} = 0.1$ and $Rf1 = 0.95$. It follows from the analysis that for the given adsorbent–eluent system it is possible to separate all components of mixture with $Rsg > 1.5$. On the other hand, the calculated value of the relative resolution r^* shows that the spacing of spots in the chromatogram is not uniform; this is because even the use of pure modifier ($c = 1$) in the last step does not permit the appropriate distance for solutes $s7$ to $s9$. It is therefore necessary to use another modifier of similar selectivity but a higher elution strength. However, if we change the $Rf1$ value to 0.75 (for the same values of $h\text{min}$ and $h\text{max}$), then all pairs of solutes remain well separated but the relative resolution is much higher ($r^* = 0.80$). A change of the initial value to $Rf1 = 0.5$ results in a much lower value of the relative resolution. In this way, trying various initial conditions, simulation of the stepwise gradient separation can be carried out, thereby obtaining the full characteristics of the separation.

For experimental verification, five coloured solutes were chosen, for which the slopes m and k_0 values were determined for the system heptane–methyl ethyl ketone[16] (Table II). The procedure for the determination of the optimum stepwise gradient programme was applied (Table III).

The theoretically determined gradient programme was then applied in stepwise gradient development. The theoretically predicted and experimental final Rfg values are compared in Table IV, and the experimental chromatogram is shown in Fig. 2. The agreement is very good. The higher values for solutes B and D can be interpreted as follows. The k_0 and m values were determined on the basis of four points and R_F values above 0.5, which limits the accuracy of estimations of k_0 and m values and then of R_F values. Moreover, there is some effect of eluent demixing, especially for low concentrations of the modifier. In the practical application of the programme, the technical possibilities of introducing small volumes of eluent fractions should be taken into account. Nevertheless, the combination of the TLC-S technique, the preliminary determination of retention–modifier concentration relationships and computer simulation and choice of the optimum stepwise gradient provides a rational approach to successful separations.

TABLE III

THEORETICAL VALUES OF *Rfg* CALCULATED BY THE COMPUTER PROGRAM AND THE OPTIMUM GRADIENT PROGRAMME SELECTED BY COMPUTER

Minimum difference in R_F values, *h*min = 0.05; maximum difference in R_F values, *h*max = 0.1. R_F value of first solute, *Rf*1 = 0.5.

Step	*Volume*	*Concentration*	*Code*	*Rfg*	*Rsg[j/(j − 1)]*
1	0.050	0.09	B	0.49	1.50 (B–D)
2	0.074	0.133	D	0.42	1.50 (D–E)
3	0.075	0.338	E	0.34	1.50 (E–H)
4	0.075	0.461	H	0.27	1.50 (H–K)
5	0.274	0.672	K	0.19	

Relative resolution *r** = 0.9999

TABLE IV

THE THEORETICALLY CALCULATED VALUES OF *Rfg* AND EXPERIMENTAL VALUES

Gradient programme selected by computer

Code	*Rfg*	
	Experimental	*Theoretical*
B	0.54	0.49
D	0.48	0.42
E	0.33	0.34
H	0.28	0.27
K	0.22	0.19

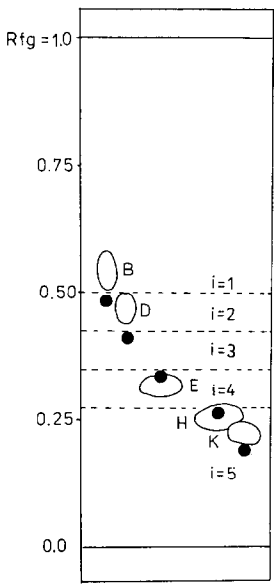

Fig. 2. Theoretical and experimental chromatograms of five solutes in the system heptane–methyl ethyl ketone on silica gel. ● = Theoretical spots of solutes; ○ = experimental spots. *i* = No. of elution step (eluent fraction).

SYMBOLS

Different symbols are used in the BASIC program for technical reasons.

$Rfg(j)$	R_F value of solute j in gradient development;
Vm	void volume of the system;
$k_0(j)$	capacity factor of solute j for unit concentration of modifier;
$k(j,i)$	capacity factor of solute j for the ith consecutive gradient step;
$c(i)$	concentration of modifier (molar or volume fraction) in the ith step;
$v(i)$	volume of eluent introduced in the ith step;
m	slope of log k *vs.* log c plot;
$Rsg(k/j)$	resolution of solute k and j in gradient development;
$w(j)$	spreading of solute spot in R_F units;
$h(j)$	difference of $Rfg(k)$ and $Rfg(j)$;
r^*	relative resolution (separation);
$y(j,i)$	distance travelled by solute j in the ith step;
$z(j,i)$	distance travelled by solute j in the ith step;
$x(j,i)$	corresponding volume of mobile phase;
$Rf(j,i)$	R_F value of solute j in the ith step;
$h$min	minimum distance between the spots of the solutes;
$h$max	maximum distance between the spots;
$sx(j)$, $sy(j)$, $sz(j)$, $sv(i)$	corresponding sums of value $sx(j,i)$, $y(j,i)$, $z(j,i)$, $v(i)$;
$p(i)$	the indicator of the last solute eluted in the ith step.

APPENDIX

```
80 'THE STEPWISE GRADIENT
90 'THE OPTIMAL PROGRAM OF GRADIENT
100 'W.MARKOWSKI
103 'DEPARTMENT OF INORGANIC AND ANALYTICAL CHEMISTRY, MEDICAL ACADEMY
105 'STASZICA 6, 20-081 LUBLIN, POLAND
110 INPUT "THE NUMBER OF SOLUTES b=";b
120 dim ko(b): dim m(b):dim k$(20,2):dim Rfg(b)
125 '=============================================================================
135 dim c(b):dim v(b)
140 '=============================================================================
155 dim k(b,b)
160 dim Rf(b,b)
165 dim x(b,b)
170 dim y(b,b)
175 dim z(b,b)
180 dim sx(b)
185 dim sy(b)
190 dim sz(b)
200 dim sv(b)
205 dim p(b)
210 '=============================================================================
220 for j=1 to b
221 INPUT "THE CODE #=";k$(j,2)
222 INPUT "THE SLOPE m=";m(j)
223 INPUT "ko=";ko(j)
224 next j
216 INPUT "THE MINIMUM DIFFERENCE IN RF VALUES hm=";hm
217 INPUT "THE MAXIMUM DIFFERENCE IN RF VALUES hx=";hx
219 INPUT "THE RF VALUES FOR FIRST SOLUTE RF1=";Rf1
235 lprint " THE PARAMETERS OF SEPARATION "
240 LPRINT:LPRINT "THE MINIMUM DIFFERENCE IN RF VALUES hm=";hm:lprint
242 LPRINT "THE MAXIMUM DIFFERENCE IN RF VALUES hx=";hx:lprint
245 lprint:lprint "THE RF VALUES OF FIRST SOLUTE Rf1=";Rf1
250 k1=int(10000*(1-Rf1)/Rf1)*.0001
300 for j=1 to b
305 c=(ko(j)/k1)^(1/m(j))
310 c(j)=int(10000*c)*.0001
315 if c(j)>1 then c(j)=1
320 next j
```

(*Continued on p. 530*)

```
325 for l=1 to b-1
330 for i=1 to b-1
335 if c(i)<=c(i+1) then go to 355
340 u=c(i)
345 c(i)=c(i+1)
350 c(i+1)=u
355 next i
360 next l
365 for i=1 to b
370 if i>=2 then c(i)=0
375 next i
385 for s=1 to b
388 if s>=2 then go to 600
390 for j=1 to b
395 k=ko(j)/(c(1)^m(j)):k(j,1)=k
400 Rf=1/(1+k):Rf(j,1)=int(10000*Rf)*.0001
405 next j
410 for j=1 to b-1
415 for l=1 to b-1
420 if Rf(l,1)<=Rf(l+1,1) then go to 440
425 u=Rf(l,1):a$=k$ (l,2):m=m(l):ko=ko(l)
430 Rf(l,1)=Rf(l+1,1):k$(l,2)=k$(l+1,2):m(l)=m(l+1):ko(l)=ko(l+1)
435 Rf(l+1,1)=u:k$(l+1,2)=a$:m(l+1)=m:ko(l+1)=ko
440 next l
445 next j
450 for j=b to 2 step -1
455 h1=Rf(j,1)-Rf(j-1,1):h1=int(10000*h1)*.0001
460 if h1=0 or  h1>=hm and h1<=hx then go to 480
465 v(1)=1-Rf(j,1):v(1)=int(10000*v(1))*.0001
470 p(1)=j
475 go to 485
480 next j
482 p(1)=1:v(1)=1
485 for j=b to p(1) step -1
488 Rfg(j)=int(10000*Rf(j,1))*.0001
495 next j
500 'The end of first step,of gradient
505 if p(1)=1 then go to 860
520 sv(1)=v(1)
525 for j=p(1)-1 to 1 step -1
528 k(j,1)=ko(j)/(c(1)^m(j)):Rf(j,1)=1/(1+k(j,1))
530 y(j,1)=v(1)*Rf(j,1)/(1-Rf(j,1))
535 z(j,1)=y(j,1)
540 x(j,1)=v(1)/(1-Rf(j,1))
545 next j
550 for j=p(1)-1 to 1 step -1
565 sy(j)=y(j,1)
570 sz(j)=z(j,1)
575 sx(j)=x(j,1)
590 next j
599 go to 850
600 sv=0
605 for i=1 to s-1
610 sv=sv+v(i)
615 next i
620 sv(s-1)=sv
621 g1=Rfg(p(s-1))-.5*(hm+hx) :g2=sz(p(s-1)-1):g3=Rfg(p(s-1))-sz(p(s-1)-1)
626 if g1>g2 then g=g1-g2
627 if g1<g2 then g=.01*g3
630 n=1-sx(p(s-1)-1):Rf(p(s-1)-1,s)   -g/n
635 k(p(s-1)-1,s)=(1-Rf(p(s-1)-1,s))/Rf(p(s-1)-1,s)
640 c=(ko(p(s-1)-1)/k(p(s-1)-1,s)):  c=c^(1/ m(p(s-1)-1))
642 if c>1 then c=1
645 c(s)=int(10000*c)*.0001
650 for j=1 to p(s-1)-1
655 k(j,s)=ko(j)/(c(s)^m(j) )
660 Rf(j,s)=1/(1+k(j,s))
665 y=Rf(j,s)*(1-sx(j)) :y(j,s)=y:next j
668 for j=1 to p(s-1)-1
670 sy=sz(j) +y(j,s):sy(j)=sy
675 next j
677 if p(s-1)-1=1 then go to 713
680 for j=1 to p(s-1)-2
685 for i=1 to p(s-1)-2
690 if sy(i)<=sy(i+1) then go to 710
695 u=sy(i):a$=k$(i,2):m=m(i):ko=ko(i)
700 sy(i)=sy(i+1):k$(i,2)=k$(i+1,2):m(i)=m(i+1):ko(i)=ko(i+1)
705 sy(i+1)=u:k$(i+1,2)=a$:m(i+1)=m:ko(i+1)=ko
710 next i
711 next j
712 if c<1 then go to 720
713 for j=1 to p(s-1)-1
714 Rfg(j)=sy(j)
715 next j
716 v(s)=1-sv(s-1)
717 go to 860
720 for j=p(s-1)-1 to 2 step -1
725 h2=sy(j)-sy(j-1):h2=int(10000*h2)*.0001
730 if h2=0 or h2>=hm and h2<=hx then go to 755
```

```
735 v(s)=1-sv(s-1)-sy(j)
740 v(s)=int(v(s)*10000)*.0001
745 p(s)=j
750 go to  758
755 next j
756 v(s)=1-sv(s-1)
758 if p(s)=1 then go to 860
760 for j=p(s-1)-1 to p(s) step -1
763 if j=0 then go to 860
764 sy(j)=int(10000*sy(j))*.0001
765 Rfg(j)=sy(j)
770 next j
775 if p(s)=1 then go to 860
780 for j=p(s)-1 to 1 step -1
783 for i=1 to s
784 k(j,i)=ko(j)/(c(i)^m(j)):Rf(j,i)=1/(1+k(j,i))
785 y(j,i)=v(i)*Rf(j,i)/(1-Rf(j,i))
790 z(j,i)=y(j,i)
795 x(j,i)=v(i)/(1-Rf(j,i))
798 next i
800 next j
805 for j=p(s)-1 to 1 step -1
810 sy=0:sx=0:sz=0
815 for i=1 to s
820 sy=sy+y(j,i)
825 sx=sx+x(j,i)
830 sz=sz+z(j,i)
835 next i
840 sy(j)=sy:sz(j)=sz:sx(j)=sx
841 next j
842 for i=1 to p(s)-2
843 for l=1 to p(s)-2
844 if sz(l)<=sz(l+1) then go to 848
845 sz=sz(l):sy=sy(l):sx=sx(l):m=m(l):ko=ko(l):a$=k$(l,2)
846 sz(l)=sz(l+1):sy(l)=sy(l+1):sx(l)=sx(l+1):m(l)=m(l+1):ko(l)=ko(l+1):k$(l,2)=k$(l+1,2)
847 sz(l+1)=sz:sy(l+1)=sy:sx(l+1)=sx:m(l+1)=m:ko(l+1)=ko:k$(l+1,2)=a$
848 next l
849 next i
850 if s>b then go to 860
855 next s
860 l=s
865 dim h(b):dim Rsg(b)
870 for j=b to 2 step -1
875 h(j)=Rfg(j)-Rfg(j-1):Rsg(j)=20*h(j)
877 Rsg(j)=int(100*Rsg(j))*.01
880 next j
885 p=1:sh=0
890 for j=b to 2 step -1
895 p=p*h(j)
900 sh=sh+h(j)
905 next j
910 sh=(sh/(b-1))^(b-1)
915 r=int (10000*p/sh)*.0001
920 lprint "=============================================================="
925 lprint "******************THE OPTIMAL GRADIENT PROGRAM ******************"
930 lprint "--------------------------------------------------------------"
935 lprint tab(11);"STEP";tab(21);"VOLUME";tab(31);"CONCENTRATION";
940 lprint tab(47);"CODE";tab(54);"Rfg";tab(61);"Rsg(j/j-1)"
945 lprint "--------------------------------------------------------------"
950 for i=1 to l
955 lprint tab(11);i;
960 v(i)=int(v(i)*1000)*.001
965 lprint tab(21);v(i);
970 c(i)=int(1000*c(i))*.001
975 lprint tab(31);c(i);
980 if i>=2 then go to 1010
985 for j=b to p(1) step -1
990 Rfg(j)=int(100*Rfg(j))*.01
995 lprint tab(47);k$(j,2);tab(54);Rfg(j);tab(61);Rsg(j);
998 lprint tab(69);k$(j,2);"/";k$(j-1,2)
1000 next j
1005 go to 1040
1010 for j=p(i-1)-1 to p(i) step -1
1020 if j=0 then go to 1040
1025 Rfg(j)=int(Rfg(j)*100)*.01
1030 lprint tab(47);k$(j,2);tab(54);Rfg(j);
1032 if j=1 then go to 1035
1033 lprint tab(61);Rsg(j); tab(69);k$(j,2);"/";k$(j-1,2)
1035 next j
1040 lprint "--------------------------------------------------------------"
1045 next i
1050 lprint "=============================================================="
1055 lprint "THE RELATIVE RESOLUTION "
1060 LPRINT "r*=";r
1065 lprint "=============================================================="
1070 end
```

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