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Computer-aided optimization of gradient multiple development thin-layer chromatography

Part II. Multi-stage development

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ABSTRACT

A theoretical model of gradient multiple development is presented as a basis for the optimization of separation by planar multi-step development and automated multiple development (AMD). A computer program for the calculation of final R_F values for multi-stage development in the gradient mode for known retention vs. eluent composition relationships is reported. The influence of various parameters on the final values of R_F is discussed. The predicted and experimental R_F values were compared and showed satisfactory agreement.

INTRODUCTION

Thin-layer chromatography (TLC) is a very popular method, applied in most analytical laboratories; the increased interest in TLC, despite the advent of HPLC, in recent years is due to its numerous advantages: simplicity, small expenditure of materials, low cost, wide choice of adsorbents [1] and solvents, diversity of techniques and equipment [2,3] and the possibility of analysing several tens of samples in parallel. Modern densitometry has made TLC an accurate and sensitive quantitative method. TLC can also be combined with other physico-chemical methods, e.g., mass spectrometry [4].

One of attractive modes of TLC is the method of multiple development, especially useful in the analysis of complex natural mixtures, e.g., plant extracts composed of numerous solutes with wide differences in polarity. Multiple development (MD) can increase considerably the resolution, R_s, owing to the reconcentration of the spots on each passage of the solvent front so that the spots become more compact, which leads to lower detection limits. It is advantageous that the process can be easily automated [automated multiple development (AMD)], which ensures good repeatability of results. The method has recently become popular in analytical practice [5] and equipment is commercially available (Camag, Muttenz, Switzerland). However, the optimization procedure is frequently carried out by the trial and error method [5] owing to the lack of a theoretical model, which would be helpful in the description of the multiple development process in its various modifications. This paper is an attempt to formulate such a physical model to describe the migration of the solute zones, their dispersion and other phenonema that may distort the development process and which can be included in the model. The model may, it is hoped, form a rational basis of various optimization procedures. The earlier derived equations for mobile phase gradients in TLC [6–8] and for migration in a two-step gradient process [9] have been utilized in the

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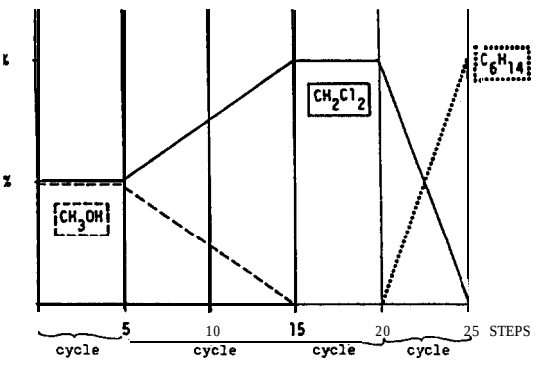
esent theoretical description of gradient multi- development.

EORETICAL

neral assumptions

We assume that the plate is developed a mber of times in the same direction and that plate is dried after each development and the lvent is completely removed from the adsor- nt layer. Further assumptions are as follows: adsorption layer has identical properties ng the whole length (thickness, phase ratio, ivity, packing density); the elution strength of mobile phase is varied according to a pro- amme (isocratic, gradient); the development tance is also varied according to a program near, stepwise or in another way); after each velopment the plate is dried and brought to h a state that the eluent delivered in the next p does not change its properties; the relation- ps between the retention of solutes and the perties of the eluents (concentration, eluent ength) are known; the solutes are not de- mposed and their loss is insignificant; the vent demixing process in the case of mixed ents is negligible to a first approximation or be taken into account in the equation; and whole elution process can be divided into les within which steps are discerned (Fig.1). a cycle we understand a number of steps in ich the same eluent type is used, differing ly in the concentration of the modifier; the ent is the solvent (containing one or more components) and the modifier is the more polar (stronger) component of the eluent.



1. Principle of partitioning of the programme into cycles steps.

In the practical realization of the multiple development process, some deviations from these simplifying assumptions may be observed. The security of identical activity of the adsorbent layer is possible only for full automation (AMD). In the case of "manual" developments, the variations in activity may cause changes in R_F values in an unpredictable manner (although for eluents that contain larger proportions of the polar modifier the effects are limited). Also, solvent demixing effects (frontal chromatography of the eluent), which tend to decrease the R_F values, are pronounced only for low contents of modifier. In each evaporation cycle some loss of more volatile sample components may occur, which may reduce the detectability, especially of trace components.

These are very general assumptions of the process of multiple development. Its variations can be classified as follows. If we assume a constant (full) development distance and a mobile phase of constant composition is used, we have the unidimensional chromatographic (UMC) technique [10]. If the distance of development in each step is longer than the previous one, we have incremental multiple development.

The model does not include the programmed multiple development (PMD) introduced by Perry *et al.* [11] when the plate is in contact with the eluent container all the time.

The following notation is used: the subscript i denotes step number; the subscript j denotes substance; y_i denotes distance travelled in step i; $s_{(h,j)}$ denotes the sum of distances travelled by solute j in h steps; $R_{F(i,j)}$ denotes the R_F value of solute j in the ith step; and $v_{e,(i,j)}$ denotes the elution volume corresponding to step i and solute j.

If we assume a programme of qualitative and quantitative composition of the eluents used in the consecutive steps, then for the purposes of computer simulation we must know the relationships between the retention of the sample components and the properties (composition) of the eluents used. For pure solvents it is simplest to give the R_F values of the solutes. On the other

hand, when binary eluents are used, it is convenient to give the parameters of the retention vs. modifier concentration plots from which the R_F values can be calculated. For normal-phase systems the equation that follows from the Snyder-Soczewinski competitive adsorption model [12] is most frequently used:

$$\log k_{(i,j)} = \log k_{0(j)} - m_{(j)} \log c_{(i)} \quad (1)$$

If the retention vs. eluent composition relationship cannot be described by equations following from the model, a polynomial of a suitable degree can be used (usually a quadratic equation is sufficient) [13]:

$$\log k_{(i,j)} = A_{(0)} + A_{(1)} \log c_{(i)} + A_{(2)}[\log c_{(i)}]^2 \quad (2)$$

$$R_{F(i,j)} = \frac{1}{1 + k_{(i,j)}} \quad (3)$$

In simulation procedures, the R_F values are introduced into the programme or are calculated by suitable subprogrammes.

When planning a multiple development programme, we introduce the number of cycles and steps. Then the development distances for the consecutive steps are to be given and then the eluent compositions used in the consecutive cycles and steps are also to be given. For the programme thus planned, the subprogramme is chosen which calculates the R_F values of solutes in the consecutive steps. Let us consider the process of multiple development for a single n-step cycle. The migration of the solutes is given by the following equations.

For the first step for which the development distance is $z_{(1)}$, the elution volume for all solutes is the same and is equal to $v_{e,(i,j)}$. This follows from the fact that all solutes are applied on the starting line at an equal distance from the lower edge of the adsorbent layer. The migration distances (from the start line) of solutes are

$$y_{(1,y)} = v_{e,1} R_{F(1,j)} \quad (4)$$

After development, the chromatogram is dried so that the spots retain their positions attained after elution. Therefore, we can write that the sum of the paths of a given solute is equal to the distance travelled in the first step. For $h = 1$ we have

$$s_{(h,j)} = \sum_{i=1}^{h=1} y_{(i,j)} = y_{(1,j)} \quad (5)$$

We carry out the next step according to the programme adopted. The development distance is now equal to $z_{(2)}$. We can have two cases: (a) the distance in the second step is greater than that in the first step (the usual case) or (b) it is smaller $[z_{(2)} < z_{(1)}]$.

(a) For the first case, $z_{(2)} > z_{(1)}$. As after the first development the solutes have various positions (distance from the start), then the elution volume in the second step is different for each solute, depending on the distance travelled in the first step. Therefore,

$$v_{e,(2,j)} = z_{(2)} - s_{(1,j)} \quad (6)$$

The migration path in the second step is

$$y_{(2,j)} = v_{e,(2,j)} R_{F(2,j)} \quad (7)$$

and the total path after two steps is

$$s_{(h,j)} = \sum_{i=1}^{h=2} y_{(i,j)} \quad (8)$$

Introducing eqns. 6, 7 and 5 into eqn. 8, we obtain an equation for the sum of migration paths after two developments:

$$s_{(2,j)} = s_{(1,j)} + [z_{(2)} - s_{(1,j)}] R_{F(2,j)} \quad (9)$$

(b) In the second case $[z_{(2)} < z_{(1)}]$, only those solutes are taken into account for which the sum of total paths is smaller than the development distance $z_{(2)}$ for the second step. For solutes which remain at their positions in the second step, we can write the following: if

$$s_{(1,j)} > z_{(2)} \quad (10)$$

then

$$v_{e,(2,j)} = 0; \quad y_{(2,j)} = 0 \quad (11)$$

and

$$s_{(2,j)} = s_{(1,j)} \quad (12)$$

For these solutes the total migration distance after the second step is the same as that after the first step. On the other hand, for solutes for

which $s_{(1,j)} < z_{(2)}$, eqns. 6 and 7 and eqns. 8 and 9 are applied,. Similar considerations are applied to further steps until the kth step. For solutes j that **fulfil** the condition $z_{(k)} > s_{(k-1,j)}$ the following equations are applied:

$$v_{e,(k,j)} = z_{(k)} - s_{(k-1,j)} \quad (13)$$

$$y_{(k,j)} = v_{e,(k,j)} R_{F(k,j)} \quad (14)$$

$$y_{(k,j)} = [z_{(k)} - s_{(k-1,j)}] R_{F(k,j)} \quad (15)$$

$$s_{(h,j)} = \sum_{i=1}^{h=k} y_{(k,j)} \quad (16)$$

and for solutes fulfilling the condition $z_{(k)} < s_{(k-1,j)}$ we apply the equations

$$v_{e,(k,j)} = 0; \quad y_{(k,j)} = 0 \quad (17)$$

and

$$s_{(k,j)} = s_{(k-1,j)} \quad (18)$$

Analogous equations are obtained for n-step development:

$$v_{e,(n,j)} = z_{(n)} - s_{(n-1,j)} \quad (19)$$

$$y_{(n,j)} = v_{e,(n,j)} R_{F(n,j)} \quad (20)$$

$$y_{(n,j)} = [z_{(n)} - s_{(n-1,j)}] R_{F(n,j)} \quad (21)$$

because $h = n$, then

$$s_{(h,j)} = \sum_{i=1}^{h=n} y_{(i,j)} = s_{(n-1,j)} + y_{(n,j)}$$

$$= s_{(n-1,j)} + [z_{(n)} - s_{(n-1,j)}] R_{F(n,j)} \quad (22)$$

For solutes which do not participate in the nth step we have

$$s_{(n,j)} = s_{(n-1,j)} \quad (24)$$

Analysing eqns. 22 and 24, we see that these are typical recurrent equations, in which the $(k-1)$th value is necessary to calculate the kth value. As the programme corresponds to n-step development, the sum of the distances travelled by a solute after n-step development is equal to the final R_F value. Taking into account the two cases , the final equations for the R_F value (R_{FG}; G = gradient) are

$$R_{FG(j)} = s_{(n-1,j)} + [z_{(n)} - s_{(n-1,j)}] R_F(n,j) \quad (25)$$

or

$$R_{FG(j)} = s_{(n-1,j)} \quad (26)$$

These equations form the basis to elaborate a computer program that simulates the multiple development process. The program, written in Pascal, is represented in Fig. 2. It allows not only for the calculation of the final R_F values but also for the graphical representation of the positions of the spots on the chromatogram. The knowledge of the R_F *vs.* i relationships for a chosen program permits the investigation by computer simulations of the effect of the number of steps, their distances and variation of eluent composition. In combination with an equation that determines the final widths of the zones [2], it permits the calculation of R_S values or other parameters that characterize the resolution and its determination [13].

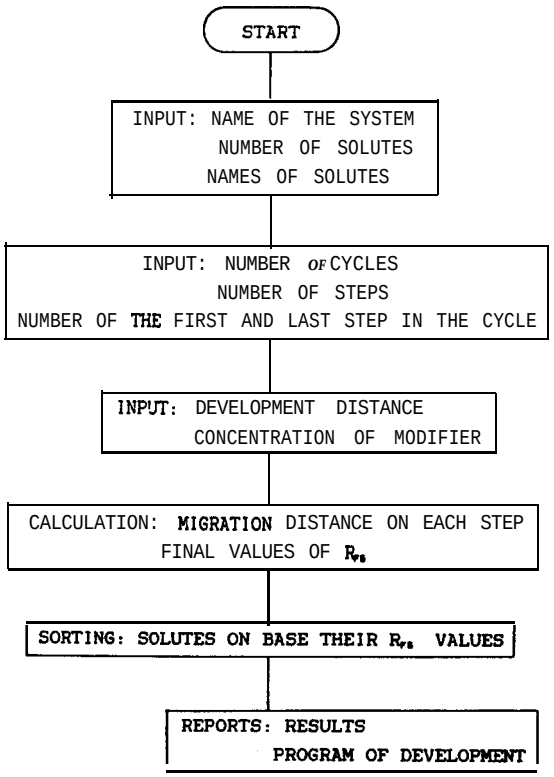


Fig. 2. Flow diagram of the computer programme for calculation of the final values of R_{FG} in multi-stage development.

EXPERIMENTAL

A horizontal sandwich chamber of the DS type [14,15] (Chromdes, Lublin, Poland) was used. Precoated plates (silica gel Si 60; Merck, Darmstadt, Germany) were activated at 100°C for 1 h and cooled in a desiccator. As eluents, solutions of diisopropyl ether in n-heptane or toluene were used; the solvents were dried over a molecular sieve (A5). The solutes were spotted on dry plates as 0.1% solutions in the eluent. When the solutes were spotted behind the solvent front, azulene was used as a marker of the mobile phase. After each development (multiple development) the plates were dried for 15 min in a stream of air. When the sample was applied from the edge of the layer, the solution of the test mixture was pipetted in a known volume into the eluent container, introduced into the adsorbent layer to the last drop and a portion of the eluent was introduced. The solutes were visible in daylight.

RESULTS AND DISCUSSION

The equation for the final R_{FG} value was verified experimentally for five-step gradient development with toluene. In the first series of experiments, the R_F values of the solutes were determined for isocratic conditions. The second experiment consisted in multiple development, applying a gradual constant increase of the development distance. The third experiment was similar to the second, except that the distance of the last step was considerably elongated. The results are presented in Tables I and II. Comparison of the simulated and experimentally determined R_F values showed satisfactory agreement. Especially in the third experiment, when the development distance of the fifth step was considerably longer, very good agreement was achieved.

In the next experiment binary eluents were used, the Snyder-Soczewinski two-parameter equation (eqn. 1) being used for the description of retention vs. eluent composition relationships. For this purpose a series of isocratic runs were carried out and the R_F values used to determine the constants of the equation, *i.e.*, the slope m and the k_0 value (corresponding to pure modifier) (Table III). The computer program was then applied to simulate the multiple development process and to compare the data with the experimental results.

TABLE I

R_{FG} VALUES OBTAINED IN FIVE-STEP DEVELOPMENT

Programme: $z_{(1)}$ = 20 mm, toluene; $z_{(2)}$ = 40 mm, toluene; $z_{(3)}$ = 60 mm, toluene; $z_{(4)}$ = 80 mm, toluene; $z_{(5)}$ = 100 mm, toluene.

Solute	$R_{FG(calc)}$	$R_{FG(exp)}$	ΔR_{FG}
4-Dimethylaminoazobenzene	0.82	0.68	0.14
Indophenol Blue	0.14	0.13	0.01
Sudan Red G	0.38	0.34	0.04
Fat Green	0.82	0.77	0.05
Fat Orange	0.74	0.78	0.04
Blue Dark	0.76	0.71	0.05
Red	0.61	0.56	0.05

TABLE II

R_{FG} VALUES OBTAINED IN FIVE-STEP DEVELOPMENT

Programme: $z_{(1)}$ = 20 mm, toluene; $z_{(2)}$ = 40 mm, toluene; $z_{(3)}$ = 60 mm, toluene; $z_{(4)}$ = 80 mm, toluene; $z_{(5)}$ = 150 mm, toluene.

Solute	$R_{FG(calc)}$	$R_{FG(exp)}$	ΔR_{FG}
4-Dimethylaminoazobenzene	0.95	0.98	0.03
Sudan Red G	0.29	0.32	0.03
Fat Green	0.73	0.73	0.00
Fat Orange	0.63	0.64	0.01
Blue Dark	0.66	0.68	0.02
Red	0.52	0.53	0.01

In the first experiment a single-cycle gradient programme was applied, the eluents being composed of diisopropyl ether and n-heptane. The results are given in Table IV. The experimental R_F values are lower than the calculated values. The cause of these discrepancies is presumably solvent demixing: as the solvent system contains two components that differ in polarity, a demixing effect is to be expected in steps 3 and 4, tending to decrease the final R_F values.

In the next experiment, a gradient programme composed of three cycles was applied; the results

ABLE III

HE PARAMETERS k_0 AND m OF THE SNYDER–OCZEWINSKI EQUATION LOG $k_{(i,j)}$ = LOG $k_{0(j)}$ – $_{(j)}$ LOG $c_{(i)}$, CALCULATED FROM A SERIES OF OCRATIC DATA FOR THE SYSTEM *n*-HEPTANE–IISOPROPYL ETHER WITH SILICA

olute	k_0	m	r
Dimethylaminoazobenzene	0.051	2.27	0.9194
dophenol Blue	0.093	2.62	0.9512
dan Red G	0.144	2.40	0.9589
t Green	0.012	3.03	0.9460
t Orange	0.037	2.38	0.9364
lue Dark	0.034	2.69	0.9515

ABLE IV

ULTIPLE DEVELOPMENT IN THE SYSTEM *n*-EPTANE-DIISOPROPYL ETHER WITH SILICA

ogramme: $z_{(1)}$ = 10 mm, volume fraction of diisopropyl her $c_{(1)}$ = 0.9; $z_{(2)}$ = 20 mm, $c_{(2)}$ = 0.7; $z_{(3)}$ = 30 mm, $c_{(3)}$ = 4; $z_{(4)}$ = 40 mm, $c_{(4)}$ = 0.3.

olute	$R_{FG(calc)}$	$R_{FG(exp)}$	ΔR_{FG}
Dimethylaminoazobenzene	0.78	0.65	0.13
dophenol Blue	0.63	0.53	0.10
t Green	0.82	0.65	0.17
t Orange	0.80	0.70	0.10
dan Red G	0.59	0.50	0.09

e presented in Table V. Also in this instance ie final R_F values are lower than the calculated alues, the main presumable cause being solvent emixing [12]; for solutes of lower R_F values $R_F < 0.6$) the discrepancies are less pronounced wing to weaker demixing effects in the lower art of the plate.

The advantages of multiple development, consting in a better distribution of the spots along ie plate and zone compression, are well known]. They can be utilized for micropreparative onal separations. The use of eqns. 25 and 26, orrected for the point of sample application and ifting of the start line to the edge of the layer, as investigated using Fat Green as the solute Table VI). The application of the sample solution from the edge (possible with the TIC hamber used [14,15]) allowed a wide starting me to be produced. By several developments

TABLE V

MULTIPLE DEVELOPMENT WITH THREE DIFFERENT ELUENTS

Programme: cycle 1, $z_{(1)}$ = 10 mm, $c_{(1)}$ = 0.9 (diisopropyl ether in heptane); $z_{(2)}$ = 20 mm, $c_{(2)}$ = 0.7; $z_{(3)}$ = 30 mm, $c_{(3)}$ = 0.4; $z_{(4)}$ = 40 mm, $c_{(4)}$ = 0.2; cycle 2, $z_{(5)}$ = 50 mm, $c_{(5)}$ = 1.0 (heptane); cycle 3, $z_{(6)}$ = 60 mm, $c_{(6)}$ = 1.0 (toluene) .

Solute	$R_{FG(calc)}$	$R_{FG(exp)}$	ΔR_{FG}
4-Dimethylaminoazobenzene	0.77	0.78	0.01
Indophenol Blue	0.45	0.44	0.01
Sudan Red G	0.49	0.50	0.01
Fat Green	0.80	0.67	0.13
Fat Orange	0.75	0.72	0.03
Blue Dark	0.74	0.63	0.11

the zone was compressed to a minimum width, which shows that the compression effects are stronger than dispersion spreading of the edges of the zone.

The results obtained indicate that the computer program for simulation in multiple development can be used for the preliminary optimization of separation conditions.

SYMBOLS

$c_{(i)}$	concentration of modifier for the ith step;
$k_{0(j)}$	capacity factor of solute j for unit concentration of modifier (pure modifier) for normal-phase systems and for $c_{(i)}$ = 0 (pure water) for reversed-phase systems;
$k_{(i,j)}$	capacity factor of solute j for the ith step;
$m_{(j)}$	slope of the log-log plot for solute j;
$R_{F(i,j)}$	R_F value for solute j corresponding to the ith concentration of modifier;
$R_{FG(j)}$	final R_F value of solute j in gradient development;
v_0	void volume (see comment on v_e);
v_e	elution volume [all values of v_0 and v_e are expressed as dimensionless magnitudes related to the void volume, v_e ($v_e = v'_e/v'_0$; $v_0 = v'_0/v'_0 = 1$)];

TABLE VI

COMPARISON OF SIMULATED AND EXPERIMENTAL ZONE WIDTHS FOR MULTIPLE DEVELOPMENT

Solute: Fat Green. Distance of development: 50 mm.

Data	Parameter	Lower edge	Upper edge
Experimental	Starting position	0 mm	6mm
	End position	43 mm	44mm
	R_F (initial)	0.00	0.60
	R_F (final)	0.86	0.88
Calculated	Starting position	Omm	6mm
	End position	43.04 mm	43.41 mm

$y_{(i,j)}$ migration distance of solute j in the ith step;

$s_{(i,j)}$ total migration distance of solute j after i steps;

$z_{(i)}$ development distance of the ith step.

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