Journal of Chromatography, 623 (1992) 139–147
Elsevier Science Publishers B V , Amsterdam

CHROM 24 386

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

I. Two-stage development

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(First received March 17th, 1992, revised manuscript received May 26th, 1992)

ABSTRACT

A theoretical model of the simplest version of gradient multiple development, the technique of two-step development, is discussed as a preliminary stage to a general theory of planar multi-step development and automated multiple development (AMD) A computer program for the calculation of final R_F values for two-step development in the gradient mode for known retention *vs* eluent composition relationships is reported Comparison of predicted and experimental R_F values shows satisfactory agreement, still better agreement is observed for the consecutive ΔR_F values

INTRODUCTION

The multiple development technique in thin-layer chromatography (TLC) is a modern concept which enables high spot capacities to be attained and is suitable for the analysis of very complex mixtures [1–4] The evolution of this method has occurred in three principal stages The first is concerned with the formulation of the experimental technique and a better understanding of the phenomena involved in the process of multiple development The second relates to attempts to automate the process the method of programmed (isocratic) multiple development The third, still in progress, is represented by the automation of multiple gradient development introduced by Burger [5]

In all techniques of multiple development, the plate is repeatedly developed in the same direction, with intermittent evaporation of the mobile phase between the consecutive developments If the layer is developed many times to the same distance with the same eluent and the plate is removed from the chamber between the stages to evaporate the solvent, the process is called unidimensional multiple chromatography, it was applied in this form by Jeanes *et al* [6] A variation of this technique, called incremental multiple development, consists in the stepwise change of the development distance which is the shortest in the first step and is then increased usually by a constant increment, the last development step corresponds to the maximum development distance If in the process of multiple development the solvent strength of the mobile phase is varied, the technique is then called gradient multiple development in either the unidimensional or the incremental version The change in the mobile phase may concern several or all steps Depending on the properties of the components of the mixture to be analysed, an increasing or decreasing gradient of solvent strength can be applied The process of

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0021-9673/92/$05 00

multiple development with an increasing gradient can be described by a model and equations reported earlier [7–12], modified to take into account intermittent evaporation of solvents Such gradients are advantageous when the mixture is to be separated into fractions containing several components In the simplest case of a decreasing stepwise gradient, the layer is developed to half the distance with a polar eluent that separates the most polar components in the lower part of the chromatogram, the less polar components are accumulated in the front area Their separation occurs in the second stage when the layer is developed to the full distance with a less polar eluent The incremental, multi-step version of this technique, with programmed, automated development and evaporation steps, is called automated multiple development [13,14], owing to flattening of the spots at each passage of the front, increased separation efficiencies are obtained and the method is considered to be the most effective and versatile technique of TLC [15]

In spite of the advantages of multiple gradient development, there is no adequate model or theory of decreasing stepwise gradient necessary for rational optimization procedures

In this paper, we present a basic theory of gradient multiple development (with computer assistance) limited to the simplest case of two-step development, carried out in a horizontal sandwich chamber [16–18] A comprehensive general theory of planar gradient multiple development will be presented in a later paper [19] It should be added that the theory of multiple development with intermittent evaporation of the solvent is inherently different from the technique of mobile phase gradients [7–12] The theory and complete programs apply also to isocratic multiple development, assuming a constant solvent strength, *i e* , k' values of individual components

THEORY

The process of two-step gradient development will be considered The following assumptions are made (i) a binary eluent is employed and the solvent strength is decreased in the second development, (ii) two steps are necessary to develop a plate, which corresponds to a total development time of 0 5–1 h, and the total migration distance is less than 10 cm, (iii) after the first development to a distance $z_{(1)}$ [$z_{(1)} < 1$], the layer is dried under vacuum, so that the first solvent is completely removed from the plate and the composition of the second solvent introduced in next step is not changed, (iv) the second development is to the total distance $z_{(2)} = 1\,0$, and (v) a quantitative retention *vs* solvent composition relationship of the solutes for the given adsorbent–eluent system is assumed

For this purpose, one of the well known equations could be applied (see ref 20 for logarithmic forms of the equations) to normal phase systems

$$k'_{(J,i)} = \frac{k^0_{(J)}}{c^{m_{(J)}}_{(i)}} \tag{1}$$

and to reversed-phase systems

$$k'_{(J,i)} = \frac{k^0_{(J)}}{10^{m_{(J)}c_{(i)}}} \tag{2}$$

where $k'_{(J,i)}$ is the capacity factor of solute J for the ith consecutive gradient step, $k^0_{(J)}$ is the capacity factor of solute J for unit concentration of modifier (volume fraction $c = 1$) for eqn 1 and for unit concentration of water [$c_{(i)} = 0$] for eqn 2 and $m_{(J)}$ is the slope of log–log plot for solute J

Consider the diagram in Fig 1, which shows the path migrated by the solute and the paths of the solvent fronts In the first step of the gradient the concentration of modifier [$c_{(1)}$] is very high and the volume of the mobile phase [$v_{(1)}$] is equal to development to a partial distance $z_{(1)}$ of less than unity For example, this distance is equal to 0 25 of the total development length The ordinate represents the migration along the plate and the abscissa the volume of eluent absorbed by the layer The void volume of the layer (v_0) is assumed to be equal to one (see Symbols) Under these assumptions the solvent front in the first step reaches only a fraction of the total development distance If that distance is 0 25 then the front of the mobile phase migrated a segment of length $z_{(1)} = 0\,25$ (in R_F units) The solute migrates a distance dependent on its properties The distance can be calculated from the equation given in our earlier papers [7–11,21]

$$y_{(J,i)} = R_{F(J,i)}z_{(1)} \tag{3}$$

where $z_{(1)}$ is the development distance in the first step [$z_{(1)} = v_{(1)}$]

The spots of the solutes are distributed along the plate according to their retention in a polar mobile

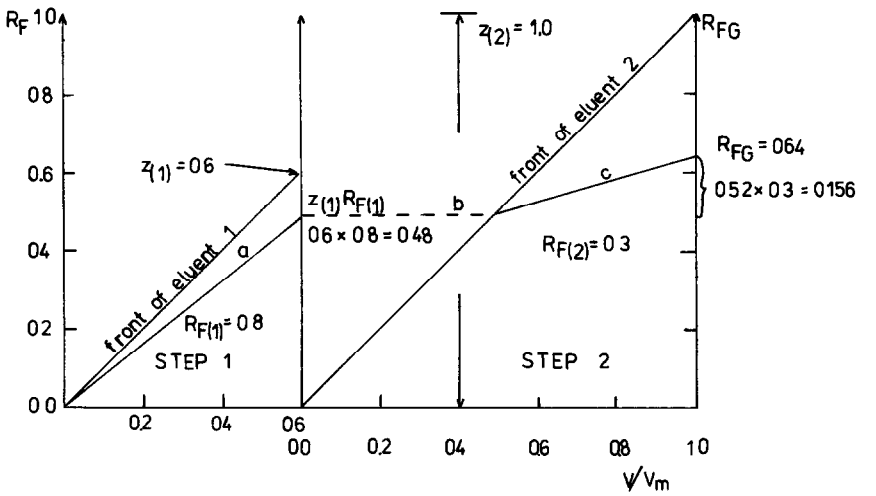

Fig 1 Schematic diagram of migration of solute in two-stage development (a) Migration of solute A during the first development, (b) during the second development the spot remains in the same place until it is reached by the front of the second eluent, (c) then the zone migrates in the second mobile phase

phase the non-polar solutes migrate near to the front but the polar solutes are separated (Fig 2)

After the first step, the plate is dried and the first eluent is removed The second development step begins again from the start line of the layer The solvent front of the second step meets the spots of solutes at different positions The volume of the mobile phase taking part in elution will be different for individual solutes and depends on their positions

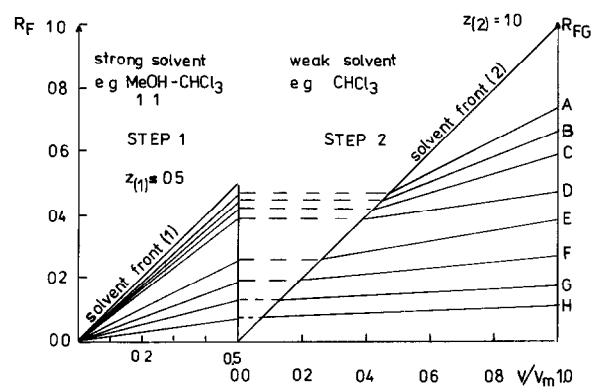

Fig 2 Migration paths of solutes A–H during a two-step development (solid lines) In the first step polar solutes E–H are separated, the less polar solutes A–D, poorly separated during the first step, are separated during the second step

after the first step The volume of solvent corresponding to the process of elution in the second step is equal to

$$v_e = 1 - R_{F(J,1)} \tag{4}$$

and the distance migrated by solute J is equal to

$$y_{(J,2)} = R_{F(J,2)}v_e = R_{F(J,2)}(1 - R_{F(J,1)}) \tag{5}$$

The total distance y migrated by solute J is

$$y_{(J)} = y_{(J,1)} + y_{(J,2)} \tag{6}$$

After substitution of eqns 3 and 5 into eqn 6 we obtain the final form of the equation which determines the final R_F value

$$R_{Fg(J)} = y_{(J)} \tag{7}$$

$$R_{Fg(J)} = z_{(1)}R_{F(J,1)} + [1 - R_{F(J,1)}z_{(1)}]R_{F(J,2)} \tag{8}$$

From eqn 8, the final R_{Fg} values of solutes in two-step gradient multiple development can be calculated This equation can be used when the values of two parameters for each solute are known, *i e*, the slope and k^0 from the log–log relationship

Eqn 8 was applied to write a computer program in GW Basic (or Pascal) The complete program is presented in the Appendix

TABLE I

ABSOLUTE SLOPES, $m_{(J)}$, AND VALUES OF CAPACITY FACTORS, $k^0_{(J)}$, OF SIX TEST SOLUTES USED IN TWO-STAGE GRADIENT DEVELOPMENT

Code	Compound	Slope, $m_{(J)}$	Capacity factor, $k^0_{(J)}$
2	2-Hydroxynaphthalene-1-azo-(4'-chlorobenzene)	1 52	0 091
3	2-Hydroxynaphthalene-1-azo-(3'-methoxybenzene)	1 67	0 023
5	Methyl red	0 86	0 885
6	Sudan III	2 25	0 008
7	Sudan IV	1 41	0 018
9	2,6-Dinitroaniline	1 00	0 279

EXPERIMENTAL

Chromatography was performed on 100 × 100 mm glass-backed plates precoated with a 0 25-mm layer of silica gel 60 (E Merck, Darmstadt, Germany) Solvents of analytical-reagent grade were purchased from Polish Reagents POCh (Gliwice, Poland) A mixture of six solutes was chromatographed to a distance of 2 5 cm in the first step and to 10 cm in the second step in Type DS equilibrium sandwich chambers [16,18], purchased from Chromdes (Lublin, Poland) During the isocratic analysis, to eliminate eluent demixing, the spotting of samples and elution were preceded by wetting the layer with a fraction of the void volume of the corresponding mobile phase The spots were detected visually In stepwise gradient experiments, fractions of the eluent of decreasing concentration of diisopropyl ether in toluene were introduced into the eluent reservoir The solvent flow was observed by means of non-retained azobenzene marker The plates were dried under vacuum obtained by a water pump The calculations were made using an IBM XT computer

RESULTS AND DISCUSSION

To illustrate the procedure and demonstrate the migration of the individual components, six compounds were chosen, their absolute slopes $m_{(J)}$ and values of capacity factors $k^0_{(J)}$ are presented in Table I

The gradient was selected as a two-step programme (Table II) with a development distance in the first step of 0 25 The concentrations in the second step were changed The computer programme calculated the R_F values for individual solutes corresponding to the chosen concentrations and the volumes of eluents in each step The final $R_{Fg(J)}$ values were then calculated by computer from eqn 8

Comparison of experimental and calculated R_F values demonstrates that for a given set of compounds whose m and k^0 values are known, it is possible to simulate the chromatographic process for various stepwise gradient programmes in the search for the optimum conditions

These data allow comparisons between predicted and experimental results Results for the tested mixture show satisfactory agreement between experimental and predicted R_{Fg} values The average errors in computer-simulated values of R_{Fg} are less than 6% Considering the method of detection of solutes and some phenomena that disturb the migration of solutes during programmed analysis, this error could be acceptable Application of densitometry as the method of detection would certainly improve the

TABLE II

GRADIENT PROGRAMME USED IN TWO-STAGE DEVELOPMENT RUNS

z = Development distance in R_F units, c = concentration of polar solvent in volume fraction

No	$c_{(1)}$	$z_{(2)}$	$c_{(2)}$	$z_{(2)}$
I	1 00	0 260	0 25	1 0
II	1 00	0 240	0 50	1 0
III	1 00	0 260	0 0[a]	1 0

[a] 100% diluent

TABLE III

COMPARISON OF EXPERIMENTAL AND SIMULATED R_{Fg} VALUES FOR TEST MIXTURE

Conditions	Code	R_{Fg} (calc)[a]	R_{Fg} (expt)[a]	R_{Fg} (graph)[a]	Error in R_{Fg} units	Error in ΔR_{Fg} units
Gradient programme I	2	0 67	0 67	0 65	0 00	0 01
	3	0 86	0 87	0 85	0 01	0 05
	5	0 36	0 30	0 33	0 06	−0 01
	6	0 89	0 94	0 90	0 05	−0 01
	7	0 92	0 96	0 93	0 04	−0 03
	9	0 52	0 59	0 59	0 01	
					Av ±0 03 (5 45%)	±0 01 (2 04%)
Gradient programme II	2	0 84	0 73		0 11	−0 05
	2	0 95	0 89		0 06	−0 02
	5	0 46	0 42		0 04	−0 01
	6	0 97	0 94		0 03	−0 00
	7	0 97	0 94		0 03	−0 02
	9	0 71	0 66		0 05	
					Av ±0 05 (7 52%)	±0 06 (1 89%)
Gradient programme III	2	0 27	0 24		0 03	0 01
	3	0 47	0 43		0 04	0 04
	5	0 14	0 06		0 08	−0 07
	6	0 44	0 45		0 01	0 00
	7	0 44	0 45		0 01	0 03
	9	0 34	0 30		0 04	
					Av ±0 02 (7 6%)	+0 01 (5 01%)

[a] Calc = values simulated, expt = values from experiment, graph = determined by graphical method [12]

precision of the R_F values obtained from gradient and isocratic measurements For the purpose of the method development and optimization of the separation, however, the errors in differences in R_{Fg} values are more important because the resolution, R_s is proportional to the difference in R_F values for adjacent pairs of spots It can be seen from Table III that the errors in ΔR_{Fg} are much smaller than those in absolute R_{Fg} values and this was observed in every instance The experimental data demonstrate that eqn 8 describes satisfactorily the chromatographic migration of solutes under conditions of two-step programmed reverse gradient elution The possibility of the prediction of R_{Fg} values with the aid of a computer is a good starting point for further optimization of analysis, *i e*, shortening the time and improving the resolution

It should be mentioned that even a simple two-step gradient development may lead to a satisfactory separation of complex mixtures, as demonstrated by Johansson [22] for plant waxes

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

$k_{(J,i)}$ Capacity factor of solute J for the ith step

$m_{(J)}$ Slope of log–log plot for solute J

$R_{F(J,i)}$ R_F value for solute J corresponding to ith concentration of modifier

APPENDIX

$R_{Fg(J)}$ Final value of R_F of solute J in gradient development

v_0 Void volume[a]

$v_{(\iota)}$ Volume of ιth step[a]

v_e Elution volume[a]

$y_{(J,\iota)}$ Migration distance of solute J in the ιth step

$y_{(J)}$ Total migration distance of solute J

$z_{(\iota)}$ Development distance of the ιth step

GW BASIC (VBASICA version 3 20c/1 40)

```
5 'W. Markowski and E. Soczewinski
15 'DEPARTMENT OF INORGANIC AND ANALYTICAL CHEMISTRY
25 'MEDICAL ACADEMY
35 'STASZICA 6, 20-081 LUBLIN
45 'POLAND
50 OPTION BASE 1
55 '================================================================
65 'MULTIPLE DEVELOPMENT   MD
75 PRINT "THIS PROGRAM USED RELATION Rm=Rmo-n *log c":PRINT
85 '----------------------------------------------------------------
90 LPRINT "MULTIPLE DEVELOPMENT ":LPRINT:LPRINT
95 PRINT "THE NUMBER OF SOLUTES"
105 INPUT "N=";N
115 DIM N$(N,10)
125 DIM KO(N):DIM M(N)
135 FOR I=1 TO N
145 PRINT "THE CODE"
150 INPUT "#";N$(I,10)
152 NEXT I
154 CLS
155 FOR I=1 TO N
160 PRINT "   # ";N$(I,10):PRINT
165 PRINT "CAPACITY FACTOR KO"
170 INPUT "KO=";KO(I)
172 PRINT "THE SLOPE m"
175 INPUT "m=";M(I)
185 PRINT "CORRECT"
195 INPUT "Y/N";Y$
205 IF Y$="N" THEN GOTO 165
210 CLS
215 NEXT I
225 '----------------------------------------------------------------
235 'THE GRADIENT PROGRAM
236  '----------------------------------------------------------------
245 PRINT "THE NUMBER OF STEPS S=2"
250 INPUT "THE NUMBER OF STEPS S=";S
255 DIM C(S):DIM Z(S)
265 FOR L=1 TO S
270 PRINT "THE STEP L=";L
275 PRINT "THE CONCENTRATION OF MODIFIER C <=1"
285 INPUT "C=";C(L)
295 PRINT "THE DEVELOPMENT DISTANCE "
305 INPUT "Z=";Z(L)
315 PRINT "CORRECT"
325 INPUT "Y/N";Y$
335 IF Y$="N" THEN GOTO 275
340 CLS
345 NEXT L:CLS
355 '----------------------------------------------------------------
365 'THE CALCULATION OF FINAL VALUES RFG
366 '----------------------------------------------------------------
370 LPRINT "THE RF VALUES IN CORRESPONDENCE TO THE STEPS OF GRADIENT"
375 DIM RG(N):DIM R(N,2)
385 FOR I=1 TO N
```

[a] All values $v_{(\iota)}$, v_e, v_0 are expressed as dimensionless magnitudes related to the void volume v_0 [$v_{(\iota)} = v_{(\iota)}/v_0$, $v_e = v_e/v_0$, $v_0 = v_0/v_0 = 1$]

```
395 FOR L=1 TO S
396 PRINT "THE STEP L=";L,
398 IF L=S THEN GOTO 645
405 K=KO(I)/(C(L)^M(I))
415 R(I,L)=1/(1+K)
418 LPRINT USING"\         \";N$(I,10);
420 LPRINT USING "       RF=#.##";R(I,L).LPRINT
425 NEXT L
435 NEXT I
445 'THE FOLLOWING CALCULATIONS ARE VALID FOR TWO STEPS DEVELOPMENT
446 '-------------------------------------------------------------------
455 FOR I=1 TO N
465 RG(I)=V(1)*R(I,1)+(1-V(1)*R(I,1))*R(I,2)
475 NEXT I
485 'THE PRINTOUT OF RESULTS
486 '-------------------------------------------------------------------
487 LPRINT
495 LPRINT "*********** THE FINAL VALUES OF RFG IN  MD **********"
505 LPRINT
515 LPRINT "THE GRADIENT PROGRAM "
525 LPRINT
535 FOR L=1 TO S
545 LPRINT USING "STEP    #";L;
555 LPRINT USING"              C=#.##";C(L);
565 LPRINT USING "                        Z=#.##";Z(L)
575 NEXT L
585 LPRINT
590 LPRINT "**********THE FINAL RFG VALUES***********"
591 LPRINT
592 LPRINT "CODE                          RFG"
593 LPRINT
595 FOR I=1 TO N
600 LPRINT USING "\      \";N$(I,10);
605 LPRINT USING "                        #.##";RG(I)
606 LPRINT
610 NEXT I
612 LPRINT "*************** END ***************"
615 CLS
620 PRINT "OTHER GRADIENT PROGRAM "
625 INPUT "Y/N";Y$
626 IF Y$="N" THEN GOTO 635
628 ERASE C,Z,RG,R
630 GOTO 245
635 END
645 PRINT "IF IN THE LAST STEP IS USED THE DILUENT THEN INPUT YES"
655 IF Y$="N" THEN GOTO 405
658 PRINT "CODE #";N$(I,10)
660 INPUT "VALUES RF IN PURE DILUENT RF=";RF
662 K=(1-RF)/RF
665 CLS
670 GOTO 415
```

TURBO PASCAL (version 6 0)

```
    PROGRAM PMD2;

USES
PRINTER,CRT;

CONST
S = 2;

VAR
N,I,J,L : INTEGER;
CONC : ARRAY [1..S] OF REAL ;
DIST : ARRAY [1..S] OF REAL;
  RF : ARRAY [1..20,1..S] OF REAL;
 RFG : ARRAY [1..20] OF REAL;
CODE : STRING [5];
  KO : ARRAY [1..20] OF REAL;
   M : ARRAY [1..20] OF REAL;
```

```
BEGIN
        WRITE (' NUMBER OF SOLUTES ');
        READLN (N);
        WRITELN ('NUMBER OF SOLUTES N=',N);
        FOR I:=1 TO N DO
        BEGIN
        WRITE('CODE OF SOLUTE # ');
        READ (CODE);
        END;
        FOR I:=1 TO N DO
        BEGIN
        WRITELN (' # ',I,CODE);
        WRITE (' KO= ');
        READLN (KO[I]);
        WRITELN(' KO= ',KO[I]:5:3);
        WRITE ('SLOPE M=');
        READLN (M[I]);
        WRITELN (' M= ',M[I]:4:2);
        END ;
        WRITELN ('     *** GRADIENT PROGRAM ***    ');
        FOR L:=1 TO 2 DO
        BEGIN
        WRITELN ('STEP L=',L);
        WRITE ('CONCENTRATION  OF MODIFIER C=0 OR C<=1 C=');
        READLN (CONC[L]);
        WRITE ('DISTANCE Z=');
        READLN (DIST[L]);
        END;
        FOR J:=1 TO N DO
        BEGIN
        FOR L:=1 TO S DO
        BEGIN
                        IF CONC [2]=0
                        THEN
                        BEGIN
                        WRITE (' GIVE VALUES OF RF FOR DILUENT ');
                        READLN ( RF[J,L] );
                        END
        ELSE
        RF[J,L]:=1/(1+KO[J]/(EXP(M[J]*LN(CONC[L]))));
        END;

        END;
        FOR J:=1 TO N DO
        BEGIN
        RFG[J]:=DIST[1]*RF[J,1]+(1-DIST[1]*RF[J,1])*RF[J,2]
        END;
        WRITELN  (LST, ' FINAL VALUES OF RFG ');
        FOR J:=1 TO N DO
        BEGIN
        WRITE (LST,' # ',I,' ',CODE,'    ',' RFG=',RFG[J]:4:2);
        END;
        CLRSCR;
END.
```


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