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PROGRAMMED MULTIPLE DEVELOPMENT

SPOT BEHAVIOR DURING SOLVENT ADVANCE; A MATHEMATICAL TREATMENT

THOMAS H. JUPILLE

Regis Chemical Co., Morton Grove, Ill. 60053 (U.S.A.)

and

JOHN A. PERRY

Consultant, Chicago, Ill. 60616 (U.S.A.)

SUMMARY

The model for spot behavior in programmed multiple development (PMD) during solvent advance can predict the number of developments necessary for maximum resolution in Mode 3. It suggests that any two spots may be completely resolved on a conventional thin-layer chromatography plate by PMD if the ratio R_F values is greater than or equal to 1.06.

INTRODUCTION

Programmed multiple development (PMD) is a technique in thin-layer chromatography (TLC). In PMD, a TLC plate is repeatedly developed with the same solvent in the same direction. Each development is longer than its predecessor. The plate is dried between developments by radiant heat or by a flow of inert gas while it is still in contact with the solvent reservoir¹ (Fig. 1).

A cycle in PMD is defined as a solvent advance (development) followed by a solvent removal (drying). The development time during the n th cycle, T_n , depends on n and on an advance unit time, t_a , according to one of three modes:

$$\text{Mode 1: } T_n = nt_a$$

$$\text{Mode 2: } T_n = \sum_{i=1}^n it_a$$

$$\text{Mode 3: } T_n = n^2t_a$$

A programmer (Fig. 2) has been designed to carry out a sequence of developments in any of these modes for unit times ranging from 10 to 100 sec (ref. 2).

The resolution and sensitivity obtained by PMD are markedly higher than those obtained on the same plates by conventional TLC³. Furthermore, the final top-to-bottom width and position of a spot on a PMD chromatogram are largely in-

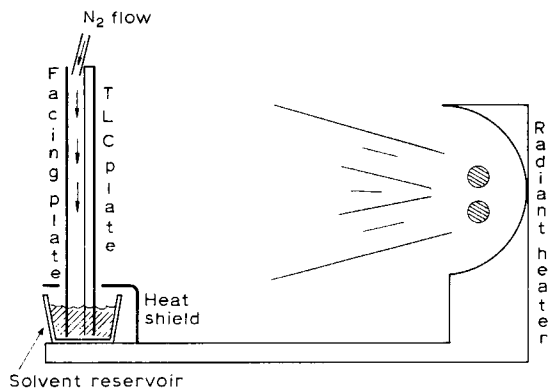


Fig. 1. Between developments, the solvent is removed by controlled evaporation. This is accomplished either by passing a flow of inert gas across the face of the TLC plate, by heating the plate, or both.

dependent of the initial width and the spot origin⁴. The need to explain and to predict the magnitude of these effects has led to a mathematical model of spot behavior during PMD. The complete model takes into account the effects on spot position and on spot width of each PMD cycle. We present here that part of the model that deals with the effects of solvent advance.

The following assumptions apply to the model presented here; all can be approximated in practice:

- (i) The effects of solvent removal are negligible.
- (ii) The entire program is carried out at constant, roughly ambient temperature.
- (iii) The distance of farthest solvent advance during any given cycle in PMD is directly proportional to the cycle number. This type of development is called Mode 3,

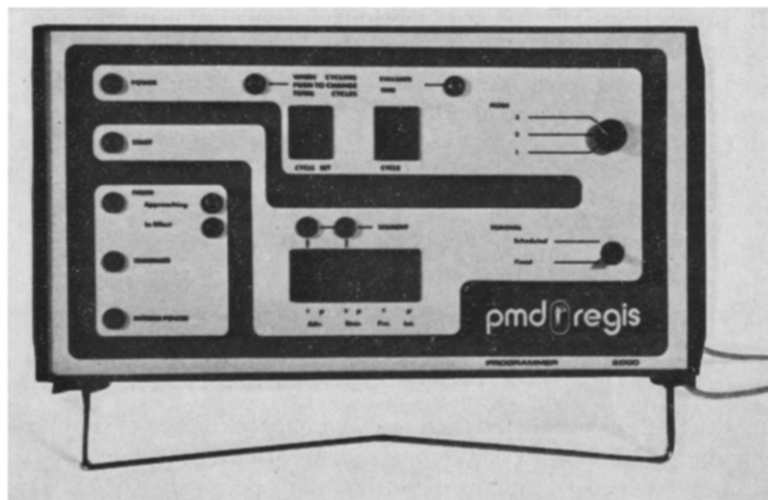


Fig. 2. The PMD programmer allows sequences of developments in any of three modes for unit times of from 10 to 100 sec.

and is approximated by setting the time for solvent advance proportional to the square of the cycle number².

We will first consider the effect on spot top-to-bottom width of successive passes of the solvent front across the spot. This part of the model, eqns. 1 through 9, is applicable to other forms of multiple development as well as to PMD. The separation of spots of similar R_F value is treated in eqns. 10 through 19. The more general question of resolution in PMD is considered in eqns. 20 through 23.

THE MODEL

On a conventional TLC plate, we may define a vertical distance X measured from the solvent reservoir level. Consider two molecules a and b of the same pure compound, but vertically displaced. These could well represent the molecules of that compound at the upper and lower edges of a single broad spot. The distances of these molecules from the solvent reservoir level are X_a and X_b , respectively (Fig. 3). The vertical distance between them is:

$$\Delta X_{ab} = X_a - X_b \quad (1)$$

The average velocity, V , of any molecule is related to the solvent velocity, V_s , by the proportionality constant R_F :

$$V = R_F V_s \quad (2)$$

The R_F is characteristic of the compound, solvent, and sorbent at a given temperature⁵.

We symbolize the positions of the molecules a and b at the end of the n th cycle as ${}_n X_a$ and ${}_n X_b$, respectively. The position of the farthest solvent advance during the n th cycle is symbolized as ${}_n X_s$.

For the n th cycle, a given molecule originates from its position at the end of cycle $(n - 1)$. Because the average velocity of the molecule is proportional to the

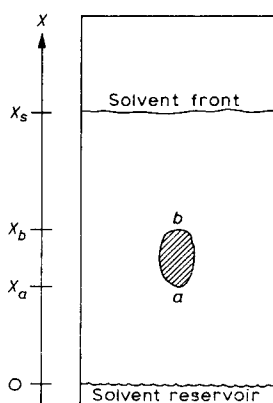


Fig. 3. Molecules at the upper and lower edges of a spot on a TLC plate may be characterized by a distance X from the solvent reservoir level.

velocity of the solvent, the distances traveled by the molecule and the solvent front are related by:

$${}_nX_a - {}_{n-1}X_a = R_F({}_nX_s - {}_{n-1}X_a) \quad (3a)$$

and

$${}_nX_b - {}_{n-1}X_b = R_F({}_nX_s - {}_{n-1}X_b) \quad (3b)$$

We re-express eqns. 3a and 3b explicitly for ${}_nX_a$ and ${}_nX_b$ and factor out ${}_{n-1}X_a$ and ${}_{n-1}X_b$:

$${}_nX_a = R_F \cdot {}_nX_s + (1 - R_F) \cdot {}_{n-1}X_a \quad (4a)$$

and

$${}_nX_b = R_F \cdot {}_nX_s + (1 - R_F) \cdot {}_{n-1}X_b \quad (4b)$$

Combining eqns. 4a and 4b with eqn. 1 gives the vertical separation ${}_n\Delta X_{ab}$ between a and b at the end of the n th cycle:

$${}_n\Delta X_{ab} = (1 - R_F) \cdot {}_{n-1}\Delta X_{ab} \quad (5)$$

If spot broadening during development is neglected, repeated explicit expression of eqn. 5 yields:

$${}_n\Delta X_{ab} = (1 - R_F)^n \cdot {}_1\Delta X_{ab} \quad (6)$$

where ${}_1\Delta X_{ab}$ is either the initial separation between two identical molecules or the initial top-to-bottom width of a single broad spot.

If we assume that a given spot broadens by an equal distance δ during each development (an assumption supported by the experimental data presented in this paper), then eqn. 5 can be modified to:

$${}_n\Delta X_{ab} = (1 - R_F)({}_{n-1}\Delta X_{ab} + \delta) \quad (7)$$

Repeated explicit expression of eqn. 7 leads to:

$${}_n\Delta X_{ab} = (1 - R_F)^n \cdot {}_1\Delta X_{ab} + \delta \sum_{i=1}^n (1 - R_F)^i \quad (8)$$

Because $1 - R_F$ is less than unity, the first term in eqn. 8 becomes negligible as the number of cycles becomes large. Therefore⁵:

$$\lim_{n \rightarrow \infty} {}_n\Delta X_{ab} = \delta \left(\frac{1 - R_F}{R_F} \right) \quad (9)$$

The number of cycles needed for the first term in eqn. 8 to decrease to 10% of its initial value is given in Table I for various values of R_F .

Eqn. 9 applies not only to PMD, but also to the technique of unidimensional multiple chromatography (UMC)⁶. In UMC a TLC plate is repeatedly developed with the same solvent in the same direction for the same length of time. This contrasts with PMD, in which each development is longer than its predecessor.

TABLE I
RELATIONSHIP BETWEEN R_F AND NUMBER OF CYCLES NEEDED FOR MINIMAL WIDTH AND FOR OPTIMUM SEPARATION

R_F	n	n_{opt}
0.9	1	1
0.8	2	1
0.7	2	1
0.6	3	1
0.5	4	1
0.4	5	2
0.3	7	3
0.2	11	4
0.1	22	9
0.05	46	19

In UMC, the center-to-center separation between two spots of similar R_F passes through a maximum value and then decreases as the number of developments increases. After a large number of developments, the spots are washed together at the solvent front. The maximum separation occurs after a number of developments, n_{opt} , given by:

$$n_{opt} = \frac{-1}{\log_c (1 - R_F)} \quad (10)$$

The value of n_{opt} for various R_F values is given in Table I.

Let us now consider two dissimilar molecules, c and d , with different R_F values of R_{Fc} and R_{Fd} , respectively. Their locations at the end of the n th PMD cycle are ${}_nX_c$ and ${}_nX_d$. If the origin of both of these spots is the same, ${}_iX$, then all distances can be measured from this origin. Such distances are indicated with a prime (Fig. 4):

$${}_nX_c' = {}_nX_c - {}_iX \quad (11a)$$

$${}_nX_d' = {}_nX_d - {}_iX \quad (11b)$$

and

$${}_nX_s' = {}_nX_s - {}_iX \quad (11c)$$

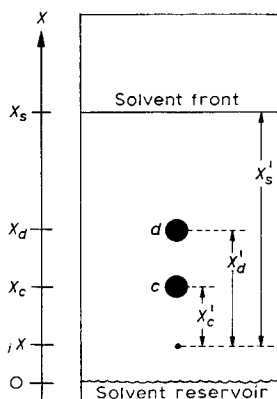


Fig. 4. Spot location in TLC may be described as a distance X' from the origin.

A typical PMD development in Mode 3 might consist of a 0.5-cm development, followed by a 1.0-cm development, then a 1.5-cm development, and so on. The limit of solvent migration during the n th cycle is given by:

$${}_nX_s' = n_1X_s' \quad (12)$$

At the end of the first cycle, the positions of the spots are related to the highest solvent front position during that cycle:

$${}_1X_c' = R_{Fc} \cdot {}_1X_s' \quad (13a)$$

and

$${}_1X_d' = R_{Fd} \cdot {}_1X_s' \quad (13b)$$

The positions ${}_1X_c'$ and ${}_1X_d'$ represent the origins for the second cycle. At the end of the second cycle, the spot positions are:

$${}_2X_c' = R_{Fc}(2{}_1X_s' - {}_1X_c') + {}_1X_c' \quad (14a)$$

and

$${}_2X_d' = R_{Fd}(2{}_1X_s' - {}_1X_d') + {}_1X_d' \quad (14b)$$

We substitute eqns. 13a and 13b into eqns. 14a and 14b and note that the result is a binomial expansion that is missing the last two terms. We can therefore rearrange eqns. 14a and 14b to:

$${}_2X_c' = \frac{{}_1X_s'}{R_{Fc}} [(1 - R_{Fc})^3 + 3R_{Fc} - 1] \quad (15a)$$

and

$${}_2X_d' = \frac{{}_1X_s'}{R_{Fd}} [(1 - R_{Fd})^3 + 3R_{Fd} - 1] \quad (15b)$$

This may be generalized to:

$${}_nX_c' = \frac{{}_1X_s'}{R_{Fc}} (1 - R_{Fc})^{n+1} + {}_1X_s' (n + 1) - \frac{{}_1X_s'}{R_{Fc}} \quad (16a)$$

and

$${}_nX_d' = \frac{{}_1X_s'}{R_{Fd}} (1 - R_{Fd})^{n+1} + {}_1X_s' (n + 1) - \frac{{}_1X_s'}{R_{Fd}} \quad (16b)$$

Substituting eqns. 16a and 16b into eqn. 1 and gathering similar terms yields an expression for the separation between c and d as a function of the number of cycles completed:

$${}_n\Delta X_{cd} = {}_1X_s' \left(\frac{1}{R_{Fc}} - \frac{1}{R_{Fd}} \right) [(1 - R_{Fc})^{n+1} - (1 - R_{Fd})^{n+1} - 1] \quad (17)$$

If the R_F values of c and d are similar (*i.e.*, if the spots are difficult to separate), then

$$(1 - R_{Fc})^{n+1} - (1 - R_{Fd})^{n+1} \ll 1 \quad (18)$$

for large values of n and

$$\lim_{n \rightarrow \infty} n \Delta X_{cd} = {}_1X_s' \left(\frac{1}{R_{Fd}} - \frac{1}{R_{Fc}} \right) \quad (19)$$

This represents an upper limit on the center-to-center separation.

Resolution is defined as the ratio of the center-to-center separation between spots, ΔX , to the average spot top-to-bottom width, W .

$$R = \frac{\Delta X}{W} \quad (20)$$

To obtain an expression for the limiting resolution in PMD, we can substitute eqn. 19 as the limiting separation and eqn. 9 as the limiting spot width into eqn. 20 and obtain:

$$R = \frac{{}_1X_s' \left(\frac{1}{R_{Fd}} - \frac{1}{R_{Fc}} \right)}{\delta \left(\frac{1 - R_F}{R_F} \right)} \quad (21)$$

where R_F is the average of R_{Fc} and R_{Fd} .

If R_{Fc} and R_{Fd} are similar in value, this equation can be rearranged and simplified to:

$$R = \frac{{}_1X_s'}{\delta} (\alpha - 1) \frac{1}{1 - R_F} \quad (22)$$

where α is the ratio of the two R_F values, also called the selectivity.

The first factor in eqn. 22, ${}_1X_s'/\delta$, is a measure of the efficiency of the TLC system (sample, solvent, adsorbent) used. The second factor, $\alpha - 1$, expresses the selectivity. The third factor, $1/(1 - R_F)$, can be related to the partition ratio, k' .

Eqn. 22 is analogous to eqn. 23 for conventional chromatography⁷:

$$R = \frac{1}{4} (N^{\frac{1}{2}}) \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'}{1 + k'} \right) \quad (23)$$

where N is the number of theoretical plates.

EXPERIMENTAL

Programmed multiple developments were carried out with a PMD programmer Model 2000 and developer Model 222 (Regis, Morton Grove, Ill., U.S.A.).

Spots were made from a benzene solution of butter yellow, Sudan red and indophenol blue (Camag, New Berlin, Wisc., U.S.A.). Commercial (Quantum) pre-coated silica gel G plates were used after being cut into 5×10 cm sections and scored with 1-mm channels located 1 cm from the long edges.

During development, the plates were clamped to matching pieces of glass plate separated by glass rods 3 mm in diameter placed at the non-immersed edges of

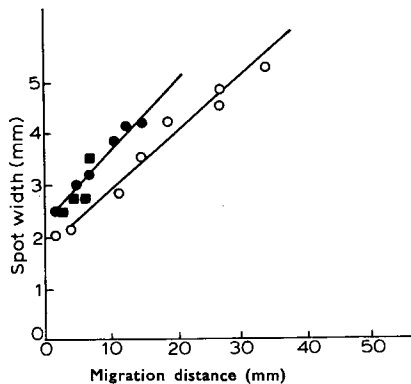


Fig. 5. Conventional TLC. Relationship between spot width and migration distance for butter yellow (○), Sudan red (●) and indophenol blue (■).

the resulting "sandwich". All developments were made with reagent grade benzene.

Three experiments were carried out:

Experiment 1. The dye solution was diluted by a factor of 500 with reagent grade benzene. Five microliters of the resulting solution was spotted 2.5 cm from the lower edge of each TLC plate. The plates were developed conventionally for varying lengths of time.

The solvent front migration distance and the locations and widths of the three major spots from the mixture were measured on each plate.

The R_F values of the spots were found to be: butter yellow, 0.53; Sudan red, 0.23; indophenol blue, 0.11.

The relationship between spot width and migration distance is shown in Fig. 5.

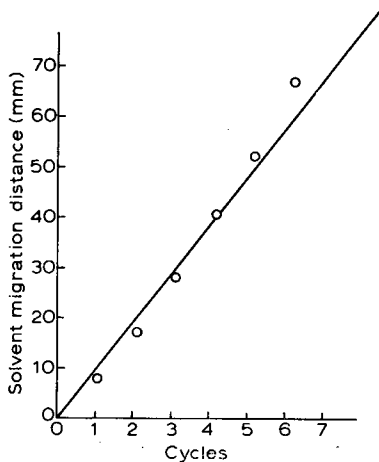


Fig. 6. PMD, Mode 3, air drying. Relationship between solvent front migration distance and cycle number.

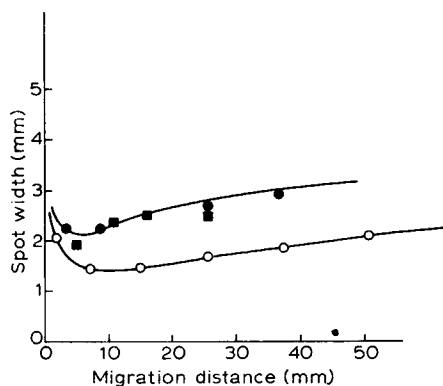


Fig. 7. PMD, Mode 3, air drying. Relationship between spot width and migration distance for butter yellow (○), Sudan red (●), and indophenol blue (■).

Experiment 2. Plates were prepared as in *Experiment 1*. The plates were repeatedly developed and air dried between developments. The first development lasted for 30 sec, the second development for 2 min, the third for 4 min 30 sec, and so on. The solvent front position and the widths and positions of the spots were measured at the end of each development.

The relationship between solvent front position and the number of developments is shown in Fig. 6, and the relationship between spot width and migration distance is shown in Fig. 7.

Experiment 3. Plates were prepared as in *Experiment 1*, but were run by PMD, Mode 3, with solvent removal by top-to-bottom nitrogen flow and minimum heat. The advance time increment was 30 sec, which gave development times of 30 sec, 2 min, 4 min 30 sec, and so on for successive cycles. The solvent front position and the width and position of each spot were measured after programs of from one to seven cycles.

The solvent front position as a function of spot width is shown in Fig. 8, and the relationship between spot width and migration distance for butter yellow is shown in Fig. 9.

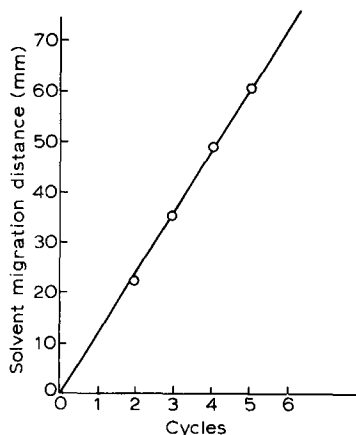


Fig. 8. PMD, Mode 3, drying by nitrogen flow. Relationship between solvent front migration distance and cycle number.

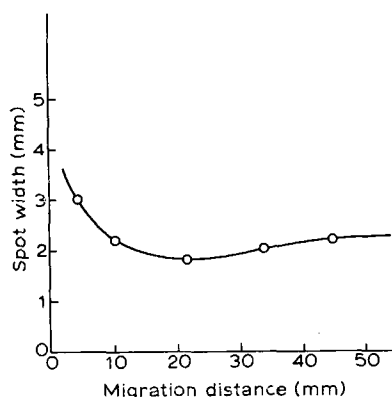


Fig. 9. PMD, Mode 3, drying by nitrogen flow. Relationship between spot width and migration distance for butter yellow.

RESULTS AND DISCUSSION

The development of this model for spot behavior during PMD solvent advance has involved a number of assumptions in order to simplify the mathematics. Although they need not be rigorously true, they should represent at least an approximation to reality. We will consider each of these assumptions in turn, along with their limits of validity.

Insignificance of solvent removal

Although solvent removal plays an important role in the normal practice of PMD, the magnitude of this role is inversely related to the speed with which the sol-

vent front recedes. When the front is made to recede quickly, the results are similar to those obtained when the plate is removed from contact with the solvent and air dried.

Ambient temperature operation

PMD solvent removal using only a flow of gas results in a temperature drop at the plate surface as the solvent evaporates. A low level of radiant heat may be applied to the plate during solvent removal in order to maintain overall ambient temperature.

Relationship between solvent migration distance and cycle number

The PMD programmer is capable of programming three modes, which are different advance time/cycle number relationships. In Mode 3, the solvent advance time is proportional to the square of the cycle number. Because the solvent advance velocity is inversely proportional to the distance between the solvent front and the solvent reservoir level (Poiseuille's Law⁸), the distance traveled by the solvent front is proportional to the square root of the time allowed for solvent advance. Accordingly, in Mode 3, the distance of maximum solvent front advance during a given cycle should be directly proportional to the cycle number. The data in Fig. 6 show this to be approximately true. The accuracy of this approximation improves as n becomes large.

Spot width is directly proportional to migration distance in conventional TLC

Despite the complex relationship among spot broadening, migration distance, and flow velocity, the data in Fig. 5 indicate that this is an accurate description of spot behavior in these experiments.

The degree of broadening during one PMD cycle is constant

Because spot migration distance per cycle approaches a constant value as n increases (Fig. 7), and because spot broadening is directly proportional to migration distance (above), this assumption is justified. A number δ may always be chosen which represents the average spot broadening per cycle during the run.

The experimental data in Fig. 7 indicate that the spot top-to-bottom width in PMD does approach a limit as n increases. This limit, however, is larger than predicted by eqn. 9.

The silica gel on the pre-coated TLC plates used in these experiments has an average particle diameter of 30μ . The classical treatment of TLC based on a Signer countercurrent distribution tank⁹ implies that no spot can be narrower than 20 to 30 particle diameters. This suggests that spot reconcentration is effective only down to approximately 0.8 to 0.9 mm in these experiments. We have, in fact, never achieved a spot narrower than about 1.1 mm (ref. 10).

Unless an adsorbent with a very small particle size, d , is used, a better approximation to the limiting spot width in PMD is given by:

$$\lim_{n \rightarrow \infty} n \Delta X_{ab} = 25d + \delta \quad (24)$$

This limit implies a minimum spot width of approximately 1 mm for small samples on commercial pre-coated plates.

UMC—the repeated same solvent, same direction on development to a given line on the plate—maximizes the center-to-center separation between spots long

before the spots approach minimum width (Table I). In the case of PMD, however, eqn. 19 predicts that the center-to-center separation between spots of similar R_F approaches an upper limit as the number of developments increases.

Eqn. 19 can be re-expressed in terms of the selectivity, α , as:

$$\lim_{n \rightarrow \infty} n \Delta X_{cd} = \frac{{}_1X_s'}{R_{Fc}} (\alpha - 1) \quad (25)$$

The distance of maximum solvent advance from the origin during the last cycle in PMD is related to the distance of solvent advance during the first cycle by:

$${}_nX_s' = n_1X_s' \quad (12)$$

Eqn. 25, therefore, can be re-expressed as:

$$\lim_{n \rightarrow \infty} n \Delta X_{cd} = \frac{{}_nX_s'}{nR_{Fc}} (\alpha - 1) \quad (26)$$

The distance ${}_nX_s'$ can be no longer than the length of the TLC plate used. Eqn. 26, therefore, predicts that the maximum center-to-center separation between two spots on a given TLC plate will be obtained when the minimum number of cycles is used to traverse the plate. The number of cycles, however, must be greater than that indicated for a particular average R_F in Table I if eqn. 26 is to be valid.

No chromatographic system or technique can resolve two components the R_F or k' values of which do not differ to some extent. We can now consider the selectivity, α , required of a system for complete resolution by PMD. By our definition of resolution in eqn. 20, we consider two spots completely resolved if $R \geq 1$. Because the limiting PMD spot width for lightly loaded samples on commercial pre-coated plates is of the order of 0.1 to 0.2 cm, we can say that complete resolution can be

TABLE II
RELATIONSHIP BETWEEN AVERAGE R_F , OPTIMUM NUMBER OF DEVELOPMENTS,
AND SELECTIVITY FOR COMPLETE RESOLUTION OF TLC SPOTS

R_F	n	α		
		${}_nX_s' = 5 \text{ cm}$	${}_nX_s' = 10 \text{ cm}$	${}_nX_s' = 15 \text{ cm}$
0.9	1	1.036	1.018	1.012
0.8	2	1.064	1.032	1.023
0.7	2	1.056	1.028	1.018
0.6	3	1.072	1.036	1.024
0.5	4	1.080	1.040	1.027
0.4	5	1.080	1.040	1.027
0.3	7	1.082	1.042	1.028
0.2	11	1.088	1.044	1.029
0.1	22	1.092	1.046	1.030
0.05	46	1.092	1.046	1.030

obtained if

$$\Delta X \geq 0.2 \text{ cm} \quad (27)$$

The substitution of the limiting separation from eqn. 26 into eqn. 27 and solving of the resulting equation yields

$$\alpha \geq 1 + (0.2) \frac{nR_{Fc}}{nX_s'} \quad (28)$$

The relationship between the average R_F of two spots and the value of α required for complete resolution by PMD is given in Table II for distances of 5 cm, 10 cm, and 15 cm.

For example, an α of 1.04 is required to resolve two spots of average R_F of 0.5 in a distance of 10 cm. Equivalent resolution by conventional TLC requires, according to eqn. 23 over 40,000 theoretical plates.

REFERENCES

- 1 J. A. Perry, *U.S. Pat.*, applied for.
- 2 J. A. Perry, K. W. Haag and L. J. Glunz, *J. Chromatogr. Sci.*, 11 (1973) 447.
- 3 J. A. Perry and L. J. Glunz, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 832.
- 4 T. H. Jupille and J. A. Perry, *J. Chromatogr. Sci.*, in press.
- 5 R. C. Weast (Editor), *Mathematical Tables from Handbook of Chemistry and Physics*, The Chemical Rubber Company, Cleveland, 1964.
- 6 J. A. Thoma, *Anal. Chem.*, 35 (1963) 214.
- 7 B. A. Karger, L. R. Snyder and C. Horvath, *An Introduction to Separation Science*, Wiley-Interscience, New York, 1973, p. 150.
- 8 B. A. Karger, L. R. Snyder and C. Horvath, *An Introduction to Separation Science*, Wiley-Interscience, New York, 1973, pp. 88, 92-94.
- 9 M. Brenner, A. Niederweiser, G. Pataki and R. Weber, in E. Stahl (Editor), *Thin-Layer Chromatography: A Laboratory Handbook*, Springer, New York, 1965, p. 88.
- 10 T. H. Jupille, unpublished results.