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## OPTIMIZATION IN THIN-LAYER CHROMATOGRAPHY SOME PRACTICAL CONSIDERATIONS\*

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### SUMMARY

The application of the results of a previous optimization theory of thin-layer chromatography is discussed with reference to problems of current interest to analytical chemists. Experimental evidence of the validity and of the practical interest of the equations derived is given. Provided the retention factors,  $R_f$ , of a series of compounds and the plate number necessary to achieve the requested degree of resolution between the spots of these compounds are known, it is easy to calculate the particle size and development length which yield the minimum analysis time.

Examples taken from either the literature or experiments described here demonstrate that, while easy separations can be performed on plates with small particle sizes in a short time and with a very small development length, difficult separations need coarser particles, greater development lengths and are slower. The most important parameter which determines the minimum analysis time is the diffusion coefficient of the solute, and the discrepancies that may occur between observed and predicted analysis times most often arise from the error in the estimation of the diffusion coefficients.

### INTRODUCTION

The availability of fine particles of either plain or chemically bonded silica has resulted in renewed interest in thin-layer chromatography (TLC). The excellent sepa-

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rations obtained on such packings led to the needless acronym HPTLC (high-performance thin-layer chromatography). In practice, an analyst carrying out TLC is attracted by the simplicity and the efficiency of the method; he is not necessarily interested in its ultimate performance but in the resolution of his own separation problems.

As early as 1976, it was demonstrated<sup>1</sup> that TLC plates prepared with very fine particles (5  $\mu\text{m}$ ) did not in many cases give better performance than regular plates. Since then the advent of nanoplates has given rise to further controversy<sup>2</sup>. In an effort to elucidate the origin of this problem we reconsidered the parameters involved in TLC. In previous papers<sup>3-6</sup> theoretical equations were developed to relate development time, efficiency and resolution to the parameters describing the plate, the chromatographic system and the separation, and a general scheme of optimization was set up.

The aim of the present work is to provide experimental evidence of the validity of these equations and to supply the analyst with the theoretical background to what is possible in TLC and what is not. A few guidelines will permit an estimate of the cost of a given analysis provided some initial conditions such as the plate number and the retention are known.

## EXPERIMENTAL

Two types of plates were used: commercially available TLC and HPTLC plates from E. Merck (Darmstadt, G.F.R.) and home-made plates. The latter were obtained by spreading a silica slurry in carbon tetrachloride on a glass plate, followed by evaporation of the solvent and activation for 1 h at 110°C. In both cases the layer thickness is 0.25 mm.

Merck plates are made with 11–15  $\mu\text{m}$  particles (TLC plates) and 5–7  $\mu\text{m}$  particles (HPTLC plates), and have a rather narrow particle size distribution. We used LiChrosorb Si 60 having similar particle sizes and particles prepared from a batch of silica supplied by I. Halász (Saarbrücken, G.F.R.). A Bacco classifier permitted the preparation of a 24–31- $\mu\text{m}$  particle sample.

LiChrosolv-type solvents were used without further purification. Sample deposition was performed with glass capillaries of internal diameter 0.1 mm. One drop of solution was spotted to prevent circular spreading of the solute on the plate. Conventional developments were carried out in a Camag chamber with a ground glass lid. Densitograms were recorded with a Zeiss PMQ II spectrophotodensitometer at a plate speed of 20 mm/min.

## THEORETICAL

The following equations were derived in previous papers<sup>3-6</sup>, to which the reader is referred for a discussion of the ranges of validity.

### *The basic equations*

As the capillary rise occurs in the plates the distance,  $z$ , between the solvent front and the solvent source is related to time,  $t$ , by

$$z^2 = kt \quad (1)$$

with

$$k = 2 k_0 d_p \frac{\gamma}{\eta} = \theta d_p \quad (2)$$

where  $k$  is the velocity constant of the solvent,  $d_p$  the particle diameter,  $\gamma$  the solvent surface tension and  $\eta$  its viscosity;  $k_0$  is a dimensionless coefficient, close to  $8 \cdot 10^{-3}$  (ref. 4). The influence of the wetting angle of the solvent on the adsorbent has been neglected in eqn. 2. This effect can be important in reversed-phase TLC.

At the end of the development, the migration distance of the solvent is  $L$ , the migration distance of the spot is  $X$  and by definition of the retention factor,  $R_F$ :

$$X = R_F L \quad (3)$$

The plate number is given by:

$$N = 16 \left( \frac{X}{W} \right)^2 \quad (4)$$

$W$  is the spot width which can be measured with accuracy only on a densitogram<sup>7</sup>. The resolution between two spots 1 and 2 of Gaussian profile is:

$$R = \frac{2 (X_2 - X_1)}{W_1 + W_2} \quad (5)$$

By analogy with the conventional definition of the plate height, Snyder<sup>8</sup> has shown that

$$N = \frac{L R_F}{H} \quad (6)$$

where  $H$  is the average height equivalent to a theoretical plate;  $H$  is a function of the development length, the retention factor and the diffusion coefficient. The plate number necessary to achieve the resolution,  $R$ , between two solutes of relative retention

$$\alpha = \frac{k'_2}{k'_1} \quad (7)$$

where  $k'_i$  is the bed capacity factor for compound  $i$

$$k'_i = \frac{1 - R_{F,i}}{R_{F,i}} \quad (8)$$

is given by:

$$N_{ne} = 16 R^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \cdot \frac{(1 + k')^2}{k'^2} \quad (9)$$

Let us assume that the local plate height is given by the conventional reduced plate height ( $h$ ) equation used in column chromatography<sup>9</sup>

$$h = \frac{B}{v} + Av^{1/3} + Cv \quad (10)$$

with

$$h = \frac{H}{d_p}, v = \frac{ud_p}{D_m} \quad (11)$$

where  $u$  is the flow-velocity,  $A$  characterizes the quality of the packing,  $C$  the resistance to mass transfer in the chromatographic bed and  $B$  the axial diffusion in that bed. Previous calculations and experiments have shown that  $A$  is approximately unity for most correctly prepared plates<sup>5</sup>, that  $C$  is usually small (0.01–0.03) and that  $B$  is given by

$$B = 2 \left[ \gamma_m D_m + \left( \frac{1 - R_F}{R_F} \right) \gamma_s D_s \right] \quad (12)$$

where  $D_m$  is the diffusion coefficient in the mobile phase,  $\gamma_m$  the tortuosity coefficient of the bed and  $D_s$  and  $\gamma_s$  the corresponding terms for the stationary phase. Two cases are possible depending on whether  $\gamma_s D_s$  is negligible or comparable in magnitude to  $\gamma_m D_m$ .

It is easy to show<sup>5</sup> that the average plate height is given by:

$$H = \frac{B}{\theta d_p} \cdot (L + z_0) + \frac{3}{2} \cdot \frac{A d_p^{5/3} \theta^{1/3}}{(2 D_m)^{1/3}} \cdot \frac{L^{2/3} - z_0^{2/3}}{L - z_0} + \frac{C \theta d_p^3}{2 D_m (L - z_0)} \log \frac{L}{z_0} \quad (13)$$

where  $z_0$  is the distance of the sample spot above the solvent level in the tank. Because  $C$  is small, in practice the third term of eqn. 13 is negligible. Fig. 1 shows plots of average plate height *versus* development length calculated for various particle sizes. These plots show the dramatic influence of the diffusion coefficient with fine particles<sup>5</sup> since for  $d_p < 10 \mu\text{m}$  we can write

$$H = \frac{B}{\theta d_p} \cdot (L + z_0) \quad (14a)$$

and  $B$  is proportional to the diffusion coefficient. The influence of  $D_m$  is less dramatic with coarse particles since for larger particle sizes we can write

$$H = b(L + z_0) + a \cdot \frac{L^{2/3} - z_0^{2/3}}{L - z_0} \quad (14b)$$

$$\text{with } b = \frac{B}{\theta d_p} \quad \text{and} \quad a = \frac{3}{2} \cdot \frac{A d_p^{5/3} \theta^{1/3}}{(2 D_m)^{1/3}}$$

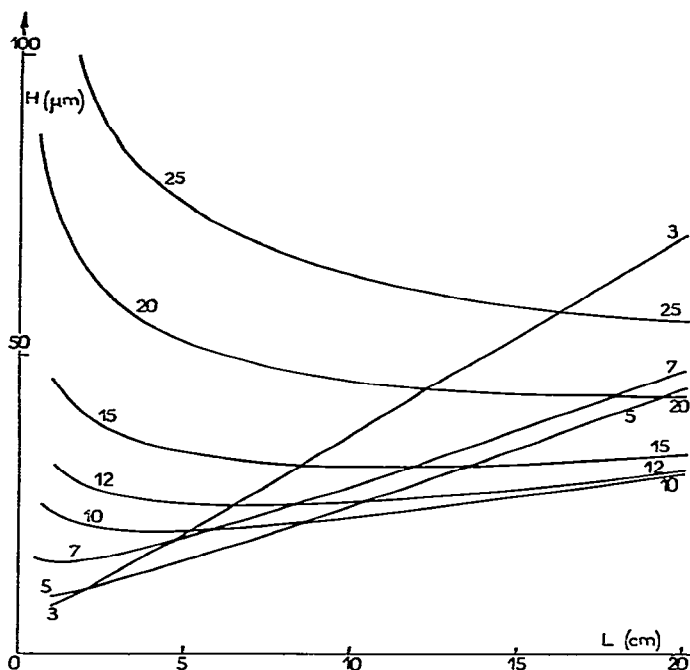


Fig. 1. Plots of the average height equivalent to a theoretical plate,  $H$ , versus development length,  $L$ , for various particle sizes.  $A = 1$ ;  $\gamma = 0.70$ ;  $D_m = 5 \cdot 10^{-6} \text{ cm}^2/\text{sec}$ ;  $C = 0.01$ ;  $R_F = 0.70$ ;  $\gamma_m D_m = \gamma D_s$ ;  $\theta = 70^\circ$  cm/sec;  $z_0 = 0.5 \text{ cm}$ . The number on each curve is the particle size.

and the coefficient  $a$  is proportional to  $D_m^{-1/3}$ . For particle sizes between 10 and 20  $\mu\text{m}$  the two terms are comparable and their variations compensate each other so  $H$  is almost constant.

### The principles of optimization

From the plots of Fig. 1 it is evident that the efficiency of coarse particle plates improves with increasing development length, while the opposite is true for fine particles. Usually development is carried out for 10 cm since the calculation of  $R_F$  is made easier and efficiency is good enough. In some cases it is worth using longer distances. This was demonstrated experimentally by Vernin<sup>10</sup> who separated essential oils with a development length of 15 cm using plates with 40- $\mu\text{m}$  particles. It must be emphasized that development on 10 cm is not the rule and that different distances may be more appropriate. The analysis time may vary widely with the particle size used<sup>1,2</sup>.

To carry out a given separation a number of theoretical plates,  $N_{ne}$ , given by eqn. 9 is needed. According to eqn. 6 we can write:

$$\frac{R_F}{N_{ne}} = \frac{H}{L} \quad (15)$$

The development length and the particle size are thus determined by the intersection with the  $H = f(L)$  curves in Fig. 1 of the straight line of slope  $R_F/N_{ne}$  passing through the origin. There are as many solutions as we have batches of the selected stationary

phase of different particle size. The best combination is that which gives the shortest analysis time or, as shown by eqns. 1 and 2, the lowest value of  $L^2/d_p^6$ .

For each problem the analyst may draw  $H$  versus  $L$  plots but this is time-consuming and until the calculation is finished he has no idea of the optimal conditions. Although these plots are quite informative, providing useful insight into the mode of TLC separations and the selection of good compromises, a more direct approach may be preferred especially in difficult cases. This is the direct calculation of the optimum conditions, using the equations derived previously<sup>6</sup>. The maximum number of plates which can be obtained in TLC is given by:

$$N_M = \frac{\theta d_p R_F^2}{2 [\gamma_m D_m R_F + \gamma_s D_s (1 - R_F)]} \quad (16)$$

The value of  $N_M$  is not the same for all solutes on the same plate but depends upon the choice of the chromatographic system and on the solute itself. It takes an infinite amount of time to generate  $N_M$  plates, so the experimental conditions should be such that  $N_{ne} < N_M$  and ideally<sup>6</sup>  $N_{ne} \approx N_{M/2}$ .

The minimum analysis time,  $t_{R,m}$ , is given by

$$t_{R,m} = \left( \frac{3A\gamma}{2R_F^3} \right)^{3/2} \frac{16 D_m}{\theta^2} N^3 \quad (17)$$

if we assume that  $\gamma_m D_m$  and  $\gamma_s D_s$  in eqn. 16 are equivalent. The corresponding optimum particle size is

$$d_{p,o} = \frac{4}{\theta R_F^2} [\gamma_m D_m R_F + (1 - R_F) \gamma_s D_s] N \quad (18a)$$

or, if we assume as before that  $\gamma_m D_m \approx \gamma_s D_s$ :

$$d_{p,o} = \frac{4\gamma D_m}{\theta R_F^2} N \quad (18b)$$

The plate number,  $N$ , in eqns. 17 and 18 is that necessary for the given separation. The development length is given by:

$$L_0 = \left( \frac{3A\gamma}{2R_F^3} \right)^{3/4} \sqrt{\gamma} \left( \frac{8D_m}{\theta R_F} \right) N^2 \quad (19)$$

In eqns. 17–19 it has been assumed that diffusion takes place in both stationary and mobile phases at the same rate so  $\gamma_m D_m = \gamma_s D_s$ . This is not always the case but it is the most unfavourable case, giving the largest values of  $d_{p,o}$  (Fig. 2), of development length and analysis time<sup>6</sup>. If calculations are performed using these equations, the resolution obtained cannot be worse than predicted. The drawback of this type of approach is that it yields a particle diameter which is not available or a development length which is impractical. When optimum particle diameter is not available, it has

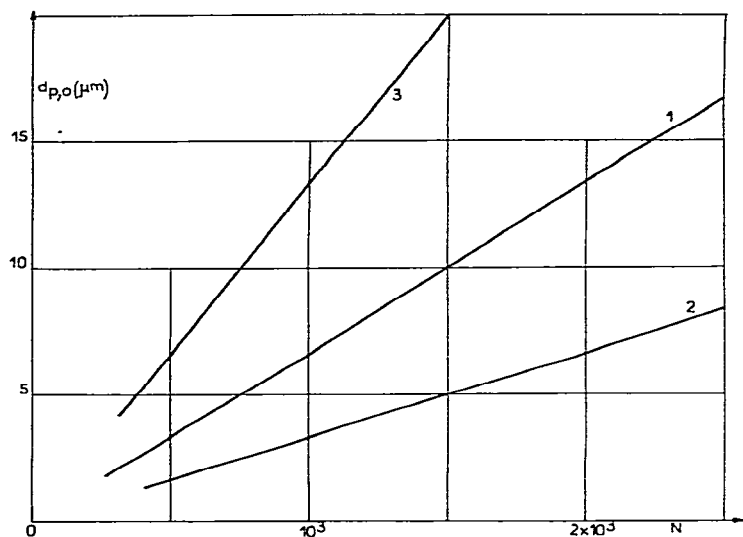


Fig. 2. Variation of the optimum particle diameter,  $d_{p,o}$ , with the number of plates,  $N$  (cf., eqn. 18a). Influence of the diffusion in the stationary phase.  $\gamma = 0.75$ ;  $D_m = 5 \cdot 10^{-6}$  cm<sup>2</sup>/sec;  $\theta = 90$  cm/sec;  $R_F = 0.5$ . Curves: 1,  $\gamma_m D_m = \gamma_s D_s$ ; 2,  $\gamma_s D_s = 0$  or  $\gamma_m D_m = \gamma_s D_s$  with  $D_m = 2.5 \cdot 10^{-6}$  cm<sup>2</sup>/sec; 3,  $\gamma_m D_m = \gamma_s D_s$ ,  $D_m = 1 \cdot 10^{-5}$  cm<sup>2</sup>/sec.

been shown that the analysis time does not increase greatly when the particles used are not more than 30 % smaller or 50 % larger than optimum<sup>6</sup>. If development length is impractical, it means either that the separation is impossible ( $L_0$  too long) or very easy ( $L_0$  too short) which should be obvious from the beginning. Thus the calculations of  $d_{p,o}$  and  $t_{R,m}$  permit a rapid selection of the optimum experimental conditions.

#### Factors influencing the optimum conditions

These factors are obvious from the equation for  $H$  (eqns. 13 and 14a). As  $C$  is often small and the third term of eqn. 13 negligible compared to the first two, the main parameters are  $R_F$ ,  $A$ ,  $D_m$  and  $\theta$ . Their influence has been discussed previously<sup>6</sup>.

As  $H$  is a function of  $R_F$ ,  $d_{p,o}$  and  $L_0$  depend on the retention. It is important to select a chromatographic system which gives a value of  $R_F$  somewhat larger than 0.33 but not much larger than 0.60<sup>6</sup>.

A decrease of the packing term  $A$  results in a decrease in the analysis time and the development length. At present, it is easy to buy or prepare plates having a packing term of unity. From our experience the influence of the binder on commercially available plates is rather small. A 10 % decrease in  $A$  would result in a 15 % reduction of the analysis time (cf., eqn. 17). We made no attempt to improve the quality of the packing, however, as we think that the loss of efficiency due to the recording systems is more important<sup>11</sup>.

The diffusion coefficient,  $D_m$ , is by far the most important factor when dealing with small particles. As soon as the mobile phase velocity has decreased below the optimum velocity which arrives for a development length which decreases rapidly with decreasing particle size<sup>6</sup>, the molecular diffusion controls the spot broadening. This is why spots in HPTLC are circular, diffusion taking place at comparable speed in the

stationary and mobile phases so that almost all spots (except for  $R_F \approx 0$ ) have the same diameter<sup>3</sup>. An increase in  $D_m$  will result in a larger value of  $H$  and the separation of closely related compounds will be more difficult. As illustrated in Figs. 2 and 3, it is much more difficult to generate large plate numbers when  $D_m$  is of the order of  $1 \cdot 10^{-5}$  cm<sup>2</sup>/sec than when it is  $5 \cdot 10^{-6}$  cm<sup>2</sup>/sec. This explains why demonstrations performed with heavy lipophilic dyestuffs yield such beautiful spots and dramatic chromatograms.

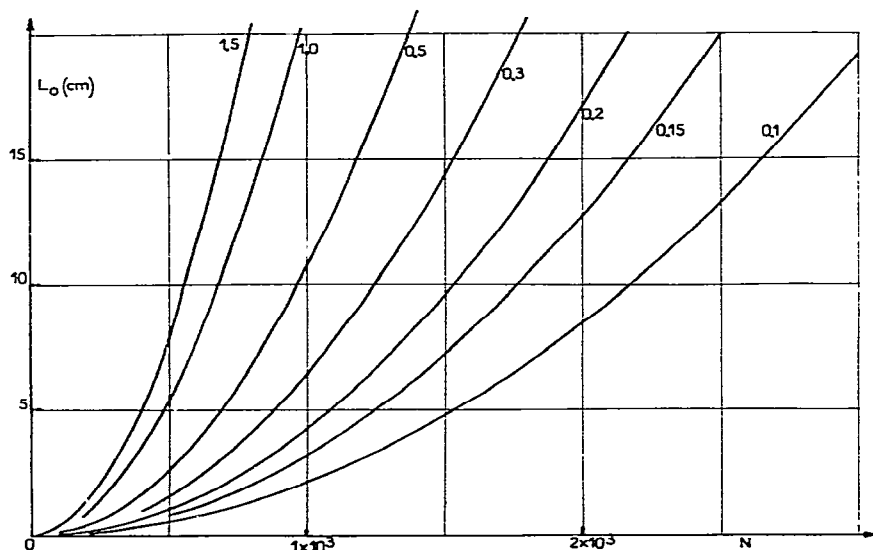


Fig. 3. Variation of the optimal development length with the necessary plate number and the diffusion coefficient (cf., eqn. 19). The number on each curve is equal to  $10^5 \cdot D_m$  (cm<sup>2</sup>/sec).  $A = 1$ ;  $\gamma = 0.75$ ;  $R_F = 0.4$ ;  $\theta = 70$  cm/sec.

Because of the scarcity of experimental data, the diffusion coefficient,  $D_m$ , is usually calculated through the Wilke–Chang equation<sup>12</sup>

$$D_m = \frac{7.4 \cdot 10^{-8} (\phi M)^{1/2} T}{\eta (V_A)^{0.6}} \quad (20)$$

where  $M$  is the molecular weight of the solvent,  $\phi$  its association constant,  $\eta$  its viscosity and  $V_A$  the molar volume of the solute. Calculation of  $D_m$  first requires the calculation of  $V_A$  and an estimate of  $\phi$ . When the density of the solute is unknown, the Le Bas method<sup>12</sup> is used to calculate  $V_A$ ; however, this is not precise and a 10% systematic error may occur between the actual and calculated  $V_A$ . The association constant,  $\phi$ , is estimated to be 1.0 for non-associated solvents such as hexane, chloroform or acetonitrile, and 2.6 for water. Great difficulties arise when a mixture of solvents is used; the calculation of  $D_m$  becomes tedious, time-consuming and inaccurate. There is a severe lack of experimental data despite the promising determination method published by Grushka and Kikta<sup>13</sup>. Unfortunately, these authors



studied diffusion of phenones in hexane, a solvent which does not move these solutes on silica gel plates, so we could not use their data.

As shown by eqns. 9 and 17, respectively, the efficiency increases and the analysis time decreases when  $D_m$  decreases. Nanoplates coated with small particles are suitable for the separation of low  $D_m$  solutes, as exemplified by the analysis of phospholipids<sup>14</sup> and the separation of digitoxin and digoxin<sup>15</sup>.

Finally, more attention in the selection of the solvent should be paid to its dynamic properties. Admittedly it is already difficult to select a development liquid while keeping the critical solute  $R_F$  between 0.35 and 0.60. Eqn. 18a, however, as well as plots of  $d_{p,o}$  versus  $N$  as shown in Fig. 4, show that for constant eluotropic strength the larger  $\theta$  is the larger the plate number is which can be achieved using particles of a given size. Thus hexane is a better solvent than octane and toluene better than benzene. Tables of velocity coefficients are available in the literature<sup>4</sup>.

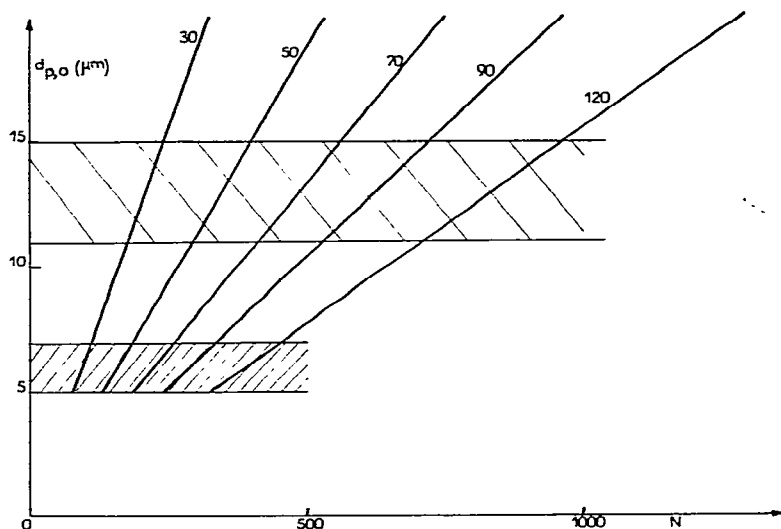


Fig. 4. Variation of the optimum particle diameter with the necessary plate number. Influence of the velocity coefficient. The number on each curve is the value of  $\theta$  (eqn. 2).  $\gamma_m D_m = \gamma_s D_s$ ;  $\gamma = 0.75$ ;  $R_F = 0.4$ ;  $D_m = 1 \cdot 10^{-5}$  cm<sup>2</sup>/sec. The hatched area corresponds to commercially available HPTLC plates (5  $\mu$ m) or TLC plates (11  $\mu$ m).

## RESULTS AND DISCUSSION

### Validity of the approach

Before we discuss the optimization problem, we must emphasize that the chromatographic contribution to  $H$  only is involved in the curves in Fig. 1. In TLC as in other chromatographic processes the variance of the solute distribution is given by:

$$\sigma_{\text{observed}}^2 = \sigma_{\text{chromatogr}}^2 + \sigma_{\text{extra column}}^2 \quad (21)$$

The extra column effects include those of injection and detection.

Excellent sample spotting is required to keep the injection effect as small as possible. Halpaap and Rippbahn<sup>16</sup> have shown that it may be as large as 20% of the total zone variance. Sample spotting using a microsyringe or glass capillaries is suitable if a very small amount of solution is delivered. Fig. 5 shows recordings of injection spots which indicate the non-Gaussian shape of the sample band. When small samples are used profiles like that in Fig. 5a make only a small contribution to the band width because they are very steep. With a width of 1.5 mm these profiles have a variance contribution of  $0.20 \text{ mm}^2$  (ref. 11), *ca.* 5% of the chromatographic variance of a spot when  $L = 10 \text{ cm}$  and  $R_F = 0.7$  (*cf.*, Fig. 1). With larger samples we obtain profiles like that in Fig. 5b with a standard deviation of 0.8 mm and a contribution of *ca.* 20%. To overcome this difficulty plates with a concentration zone are now available<sup>17</sup>.

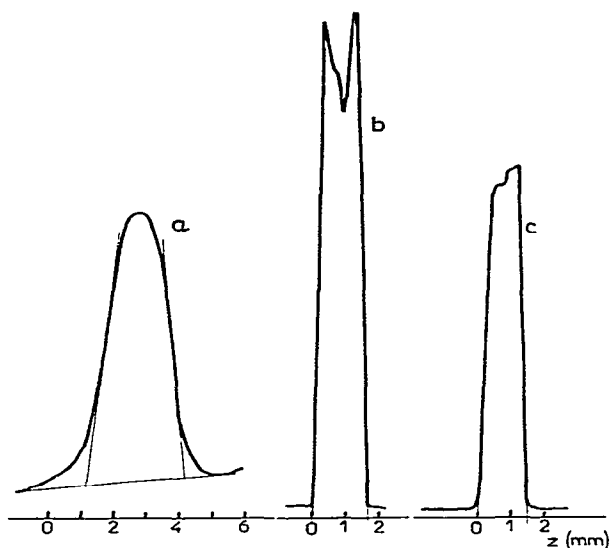


Fig. 5. Recording of sample spots in reflection mode. a, Solute pyrene, detection by fluorescence,  $\lambda_{ex}$  280 nm,  $\lambda_{em}$  375 nm, plate travel 0.5 mm/min, recorder chart speed 5 mm/min. b and c, Solute bis(chlorophenyl)triazene, UV detection,  $\lambda$  366 nm, plate travel 0.5 mm/min, recorder chart speed 10 mm/min.  $Z$  = Actual distance on the TLC plate. b, Microsyringe; c, micropipette.

Provided a good spectrophotodensitometer is used the detector variance contribution is not too large, but with many instruments currently available it may still represent a very important contribution. It will be discussed in a forthcoming paper<sup>11</sup>.

The comparison between theoretical curves and experimental data made by Halpaap and Krebs<sup>17</sup> is very interesting. As seen in Fig. 6, there is a striking agreement which can be accounted for by assuming negligible extra-bed contributions. No information was given about the particle size, but Merck TLC plates are made with 11–15  $\mu\text{m}$  particles and HPTLC plates with 5–7  $\mu\text{m}$  particles<sup>4</sup>. The chemical formula of the lipophilic dyestuff used was also not given, but it is obviously a high-molecular-weight molecule and  $D_m$  should lie between  $5 \cdot 10^{-6}$  and  $10 \cdot 10^{-6} \text{ cm}^2/\text{sec}$ . A long

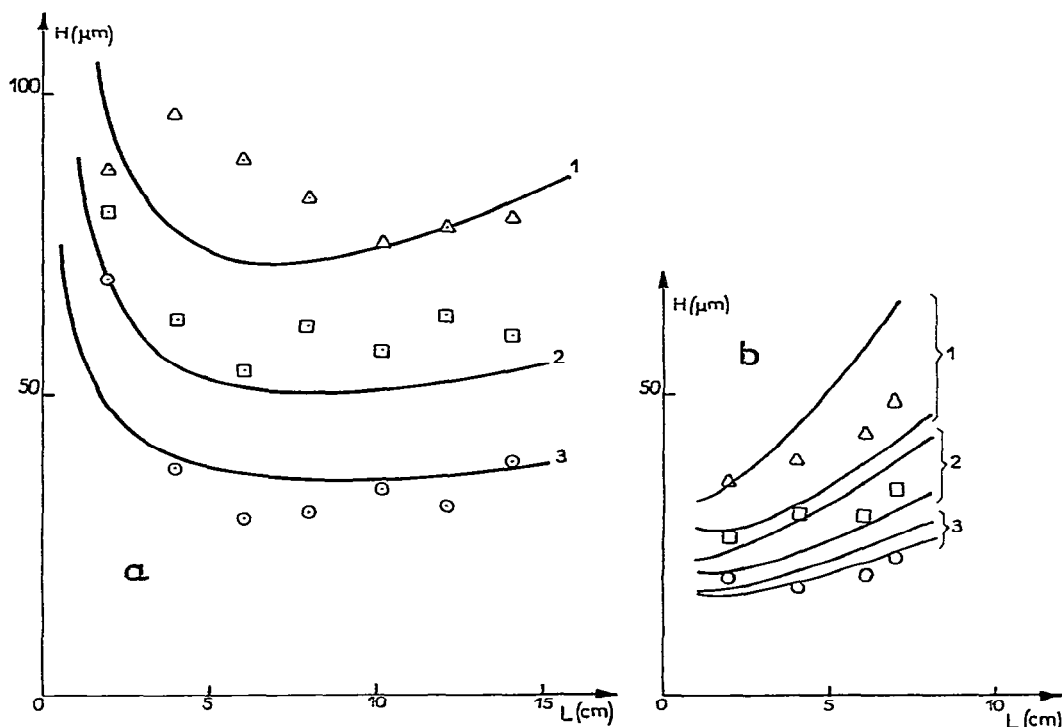


Fig. 6. Plots of  $H$  versus  $L$  for data from ref. 17 (Table XIII). Dyestuffs:  $\circ$ , violet ( $D_m = 6 \cdot 10^{-6}$  cm<sup>2</sup>/sec.  $R_F = 0.75$ ,  $C = 0.10$ );  $\square$ , green ( $D_m = 6 \cdot 10^{-6}$  cm<sup>2</sup>/sec.  $R_F = 0.45$ ,  $C = 0.20$ );  $\triangle$ , blue ( $D_m = 4 \cdot 10^{-6}$  cm<sup>2</sup>/sec.  $R_F = 0.17$ ,  $C = 0.20$ ). Experimental points and curves calculated with  $\theta = 70$  cm/sec.  $\gamma = 0.75$ ,  $A = 0.80$  and  $d_p = 15$   $\mu$ m for TLC plates (a);  $A = 1.0$  and  $d_p = 7$   $\mu$ m for HPTLC plates (b). Curves: 1, blue; 2, green; 3, violet. The lower curves in b correspond to the assumption that  $\gamma_v D_v = 0.5 \gamma_m D_m$ . All others to  $\gamma_v D_v = \gamma_m D_m$ .

development was not performed on the HPTLC plate as Halpaap<sup>16,17</sup> was well aware that small particles yield good efficiency only for short development distances.

A glance at the published chromatograms<sup>16,17</sup> shows that some separations are best performed on TLC plates rather than on HPTLC ones. The explanation is provided by the theoretical curves of Fig. 1. Easy separations can be performed with small particles over very short distances; difficult separations must be performed with coarser particles and longer development.

### Examples of application

Consider first an easy separation: pyrene from chrysene. According to previously published data<sup>18</sup>, the capacity factors with the system *n*-hexane and silica are respectively 4.11 and 6.61. Calculation of the plate number necessary to achieve the separation yields 150 plates for a resolution of unity or 230 plates for  $R = 1.25$  (cf. eqn. 9). A very short development on a plate coated with small particles will be convenient. This is demonstrated in Fig. 7 which shows densitograms obtained after 2 cm development on plates coated with particles of different size. The resolution obtained with 5- $\mu$ m particles allows quantitation of very small amounts of one solute in

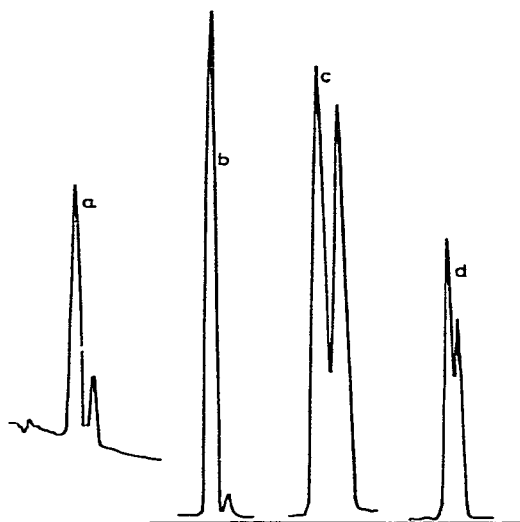
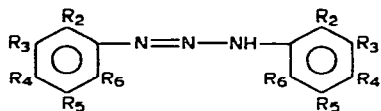


Fig. 7. Separation of pyrene and chrysene. Development length: 2 cm. Detection: fluorescence,  $\lambda_{ex}$ , 254 nm,  $\lambda_{em}$ ,  $> 375$  nm. a and b, Nanoplate silica ( $5 \mu\text{m}$ ), resolution 1.2; c, silica ( $11 \mu\text{m}$ ), resolution 0.75; d, silica ( $40 \mu\text{m}$ ), resolution 0.55. Developing solvent: hexane.

a mixture of both. It becomes poorer as the particle size increases while the development length is kept at 2 cm; at the same time the development time decreases like 7, 2 and 1.

Consider now the more difficult separation of bis(chlorophenyl)triazenes<sup>19</sup>. The retention factors are given in Table I. In order to separate the isomers III–V we need about 1000 plates for  $R = 1$ , 1500 plates for  $R = 1.25$ . Since the separation was performed with carbon tetrachloride,  $\theta = 46^\circ$ . The calculation of the diffusion coefficient using the Wilke and Chang formula<sup>12</sup> gives  $D_m = 9 \cdot 10^{-6} \text{ cm}^2/\text{sec}$ , with an error of probably less than 10%. Assuming  $\gamma_m D_m = \gamma_s D_s$  we can draw curves similar to those in Fig. 1, using the exact values of the parameters pertaining to the problem.

TABLE I  
TRIAZENES STUDIED



No.	Compound	Structure					$R_F$	$k'$
		$R_2$	$R_3$	$R_4$	$R_5$	$R_6$		
I	Bis(4-chlorophenyl)triazene	H	H	Cl	H	H	0.10	9
II	Bis(3,5-dichlorophenyl)triazene	H	Cl	H	Cl	H	0.28	2.6
III	Bis(2,4-dichlorophenyl)triazene	Cl	H	Cl	H	H	0.58	0.72
IV	Bis(2,3,4-trichlorophenyl)triazene	Cl	Cl	Cl	H	H	0.67	0.49
V	Bis(2,3,5-trichlorophenyl)triazene	Cl	Cl	H	Cl	H	0.78	0.28

For the sake of simplicity, only curves corresponding to a few particle sizes are represented in Fig. 8. According to eqn. 15, the two straight lines of slopes  $R_F/N$  equal to  $7.2 \cdot 10^{-4}$  and  $5 \cdot 10^{-4}$ , respectively, represent the conditions required to generate 1000 and 1500 plates respectively with a compound having  $R_F = 0.72$ .

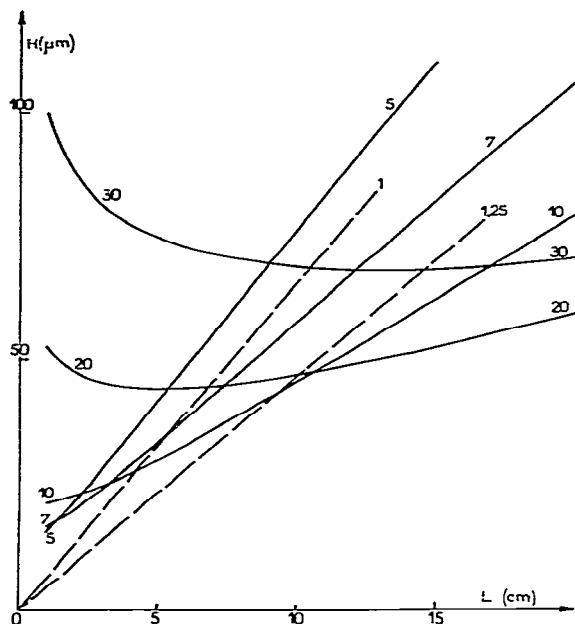


Fig. 8. Conditions for achieving resolution of bis(chlorophenyl)triazene isomers. Plots of  $H$  versus  $L$  and lines of slope  $R_F/N_{\text{ne}}$  (for resolutions of 1 and 1.25 respectively).  $A = 1$ ;  $\gamma = 0.70$ ;  $D_m = 9 \cdot 10^{-6} \text{ cm}^2/\text{sec}$ ;  $R_F = 0.70$ ;  $\gamma_m D_m = \gamma_s D_s$ ;  $\theta = 46 \text{ cm/sec}$ ;  $z_0 = 0.5 \text{ cm}$ .

The most important feature of Fig. 8 is that there is no intersection with the 5- $\mu\text{m}$  curve and consequently the complete separation of the bis(chlorophenyl)triazenes would not be possible on such plates. Different experimental procedures are possible, as summarized in Table II. There is good agreement between the theoretical and experimental results, especially when one considers the errors involved in the determination of the experimental resolution.

TABLE II  
SEPARATION OF BIS(CHLOROPHENYL)TRIAZENES

Development length and particle size necessary to achieve a given resolution. Theoretical predictions and experimental results.

$d_p$ ( $\mu\text{m}$ )	Theoretical		Experimental	
	$R = 1.25$	$R = 1$	$R = 1.25$	$R = 1$
30	14.5	10.5	14	11.5
20	10	7	13.5	10.5
10	9.5	4.5	9.5	5
7	Impossible	5.5	—	—
5	Impossible	Impossible	Impossible	Impossible

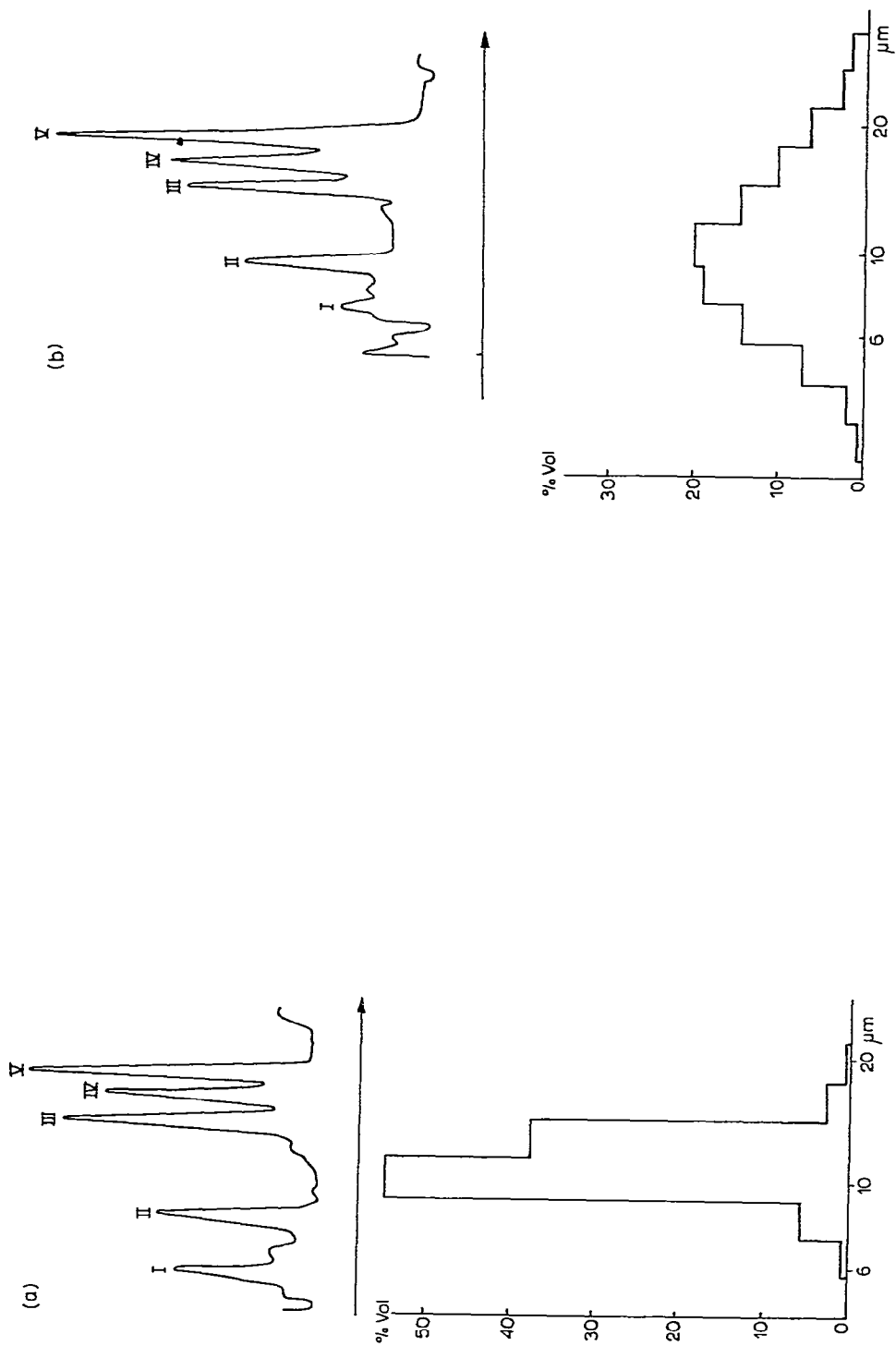


Fig. 9. Separation of bis(chlorophenyl)triazene isomers on different batches of 11-μm silica. The size distributions of the silica samples used to prepare the plates are given below the chromatograms.

As shown by Fig. 1 and evidenced by Table III, with coarse particles  $H$  remains nearly constant for development lengths between 8 and 25 cm and consequently the efficiency increases in proportion to the development length. The results obtained with very small particles are slightly better than predicted, possibly because it is easier to prepare "good" plates, with  $A = 1$ , from small particles than from coarse ones. Another reason is that the spectrophotodensitometer gives a lower contribution to the spot width when the development is long. It is obvious that the complete resolution of the mixture is impossible with very small particles.

TABLE III  
SEPARATION OF BIS(CHLOROPHENYL)TRIAZENES  
Experimental data on plates of silica Si 60 having different particle sizes.

Particle size ( $\mu\text{m}$ )	Development length (mm)	Resolution	
		III/IV	IV/V
25-31	90	0.64	0.73
	110	0.85	0.87
	120	1.03	1.04
	130	1.13	1.22
20	105	0.89	1.01
	130	1.06	1.22
11	55	0.93	1.08
	95	1.13	1.25
5	35	0.78	0.70
	40	0.88	0.88
	50	0.86	0.90
	80	0.88	0.95

It must be emphasized also that the width of the particle size distribution is very important. This is illustrated in Fig. 9 which shows the same separation achieved using two different batches of 11- $\mu\text{m}$  silica particles. When the size distribution is large or when a small amount of particles of different diameter is present the separation ability of the plate is dramatically reduced.

We consider now two other examples taken from the literature. Bidlo-Igloy<sup>20</sup> carried out the separation of the three monochloroaniline isomers in a U-chamber, after a 2-cm development with toluene ( $\theta = 77$ ) as solvent. Linear  $R_F$  values calculated from the circular ones published are: 2-chloroaniline,  $R_F = 0.41$ ,  $k' = 1.44$ ; 3-chloroaniline,  $R_F = 0.24$ ,  $k' = 3.1$ . From separate calculations as explained above, we obtain  $D_m = 1.9 \cdot 10^{-5} \text{ cm}^2/\text{sec}$  and  $N_{nc} = 100$  ( $R = 1$ ). A plot of  $H = f(L)$  (cf., Fig. 10) shows that the separation is very easy using particles of small size and with a short development length, in agreement with published data. The predicted development length is 1.5 cm with 5- $\mu\text{m}$  particles for  $R = 1$ . It would be about the same with 10- $\mu\text{m}$  particles, however, and ca. 5 cm with 15- $\mu\text{m}$  particles.

The last example is an analysis of aflatoxins<sup>21</sup>. The separation is difficult and not complete during the first run. Aflatoxins are high-molecular-weight molecules ( $\text{C}_{17}\text{H}_{12}\text{O}_7$  and  $\text{C}_{17}\text{H}_{12}\text{O}_6$ ) and  $D_m = 5 \cdot 10^{-6} \text{ cm}^2/\text{sec}$  in the developing solvent chloroform-acetone (90:10). If we assume that  $R_F = 0.5$ , the  $H = f(L)$  plots show that, as for the separation of bis(chlorophenyl)triazenes, the aflatoxin separation is

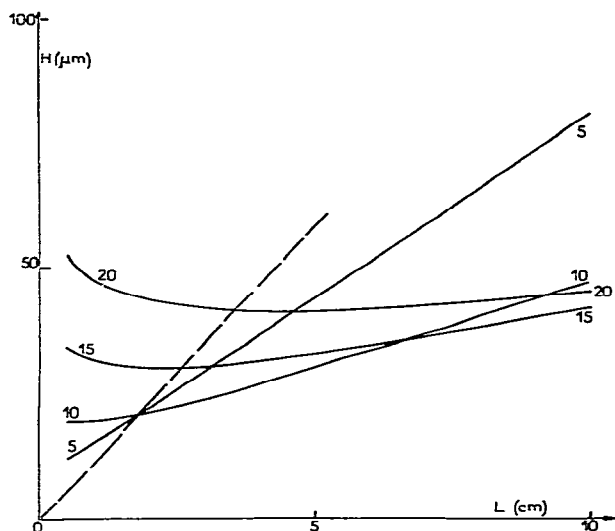


Fig. 10. Plots of  $H$  versus  $L$  used to select the optimum conditions for the separation of chloroaniline isomers according to ref. 20.  $\gamma_s D_s \approx 0$ ;  $D_m = 1.9 \cdot 10^{-5}$  cm<sup>2</sup>/sec;  $A = 1$ ;  $C = 0.01$ ;  $\gamma = 0.75$ ;  $\theta = 77$  cm/sec.

impossible on 5- $\mu$ m particle plates since the  $R_F/N_{ne}$  line does not intercept the 5- $\mu$ m or 7- $\mu$ m curve. This result illustrates the fact discussed above, that there is a limit to the number of plates that one can expect from a thin-layer chromatographic plate (*cf.*, eqn. 16). In this case again 10- or 20- $\mu$ m particles would give the best results.

### Practice of optimization

In practice, optimization is possible only if the analytical aim can be stated precisely, which means that some characteristics of the separation must be known. For unknown mixtures some qualitative information must first be acquired and then measurements made of  $R_F$ .

Suppose  $N_{ne}$  is known, as for the separation of 2,5- and 2,4-dimethylphenols with benzene on silica which requires 600 plates<sup>22</sup>. This separation is relatively easy. The elutropic strengths of toluene and benzene are similar, but the velocity coefficient is 70 for toluene compared with 62 for benzene;  $D_m$  is  $1.5 \cdot 10^{-5}$  cm<sup>2</sup>/sec<sup>12</sup>. We may calculate  $d_{p,0}$  and  $L_0$  using eqns. 18a and 19, or examine Fig. 3 which has been drawn using the numerical values of the parameters corresponding to this problem. We can conclude that 20- $\mu$ m particles are particularly suitable with a development length of 12 cm and the analysis time will be 800 sec.

Optimization is especially valuable in routine analysis where time is important. The separation of  $\alpha$ -,  $\beta$ - and  $\gamma$ -tocopherols is an example<sup>23</sup>. It appears easy as the  $R_F$  values of the three compounds are very different in hexane-chloroform (50:50) ( $\theta$  *ca.* 80):  $\alpha$ -tocopherol,  $R_F = 0.74$  ( $k' = 0.35$ );  $\beta$ -tocopherol,  $R_F = 0.47$  ( $k' = 1.13$ );  $\gamma$ -tocopherol,  $R_F = 0.23$  ( $k' = 3.35$ ). A resolution of unity would be achieved with about 150 plates (*cf.*, eqn. 9). Tocopherols ( $C_{27}H_{46}O_2$ ) are large molecules and, as we may have expected,  $D_m$  is low, *ca.*  $5 \cdot 10^{-6}$  cm<sup>2</sup>/sec. The plots in Figs. 2 and 4 show that the optimum particle size would be *ca.* 2.5  $\mu$ m and the corresponding development



length 1 cm. With a development length of 2.5 cm, the optimum particle size is *ca.* 5  $\mu\text{m}$ . In this particular case, it is very important to shorten the analysis time since tocopherols are very sensitive to oxidation and photolysis by UV light. The separation is illustrated in Fig. 11.

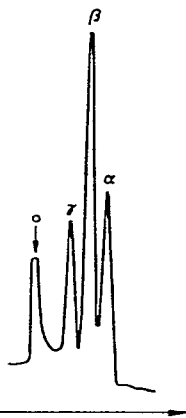


Fig. 11. Separation of tocopherols. Solvent: hexane-chloroform (50:50). Development length: 2.5 cm. Detection: UV absorption, 306 nm, reflection mode. o = Unknown.

An example for which we had no previous information about the characteristics of the separation was the analysis of furocoumarins in bergamot oil. The aim is to separate bergapten, which is considered to be carcinogenic, from other coumarins. The optimization procedure was as follows. First a series of 2-cm developments was carried out on nanoplates to check the retention factors and optimize them if necessary with a solvent of maximum  $\theta$ . When these factors and the necessary plate number are approximately known, and a check on plots like those in Fig. 4 permit an estimate of the optimum conditions for the separation. With large molecules we use graphs corresponding to  $D_m$  between  $5 \cdot 10^{-6}$  and  $8 \cdot 10^{-6} \text{ cm}^2/\text{sec}$ , with small molecules graphs corresponding to  $D_m$  between  $1 \cdot 10^{-5}$  and  $1.5 \cdot 10^{-5} \text{ cm}^2/\text{sec}$ . Finally, the separation of bergapten is performed on cyclohexane-ethyl acetate (70:30) with 20- $\mu\text{m}$  particles and a development length of 12 cm (Fig. 12).

## CONCLUSIONS

With the help of these simple optimization rules, TLC is greatly facilitated. Analysis times are reduced and quantitation is made easier with the equipment available. At the same time, these results may be achieved with a minimum of trials and previous measurements following a simple procedure which is easy to normalize. This approach is of course independent of the nature of the particles, silica, chemically bonded silica, cellulose, polyamides, etc., except that for cellulose and polyamide it may be more difficult to find the desired particle size. It is also independent of the nature of the solvent mixtures used as long as they are reproducible. Faster analyses permit the use of simpler solvent mixtures which always improves the reproducibility of the analysis.

Finally, this work also illustrates the fact that any general comparison between

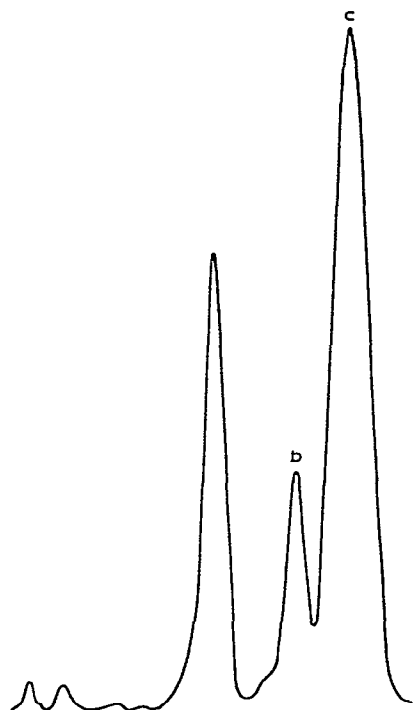


Fig. 12. Separation of citropten (c) from bergapten (b) in bergamot oil. Detection: fluorescence,  $\lambda_{ex}$  280 nm,  $\lambda_{em}$  375 nm. Development length: 12 cm on 20- $\mu$ m silica. Solvent: cyclohexane-ethyl acetate (70:30).

TLC and HPTLC is meaningless. There are cases (dyestuffs, pyrene-chrysene, chloroanilines, tocopherols) where the best results are obtained with nanoplates or TLC plates made from 5- $\mu$ m particles. In other cases [bis(chlorophenyl)triazenes, aflatoxins, 2,4- and 2,5-dimethylphenols, citropten-bergapten] better performances are obtained with normal TLC plates or plates prepared from 10- or 20- $\mu$ m particles. When TLC gives better performances than "high-performance TLC" this merely highlights the inadequacy of the name and that the distinction between the two techniques is irrelevant and ill-founded. In reality there is only one technique, which can employ a variety of materials among which the analyst has to choose the most suitable. Generally, small molecules having large diffusion coefficients are best analyzed on large particles, while large molecules having small diffusion coefficients are easier to separate on plates made from small particles. However, the difficulty of the separation also has some bearing on the choice of the optimum particle size; other things being equal, difficult separations have to be carried out on coarser particles.

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