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STUDY OF THE PERFORMANCES OF THIN-LAYER CHROMATOGRAPHY

SPOT CAPACITY IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The spot capacity in thin-layer chromatography (TLC) is the number of spots resolved with a resolution of unity that can be placed between the sample spot and the spot of a non-retained compound. This is more difficult to calculate than the equivalent peak capacity in column chromatography as the plate height in TLC is a complex function of the characteristics of the solvent used and the plate, including particle size and development length. An iteration method is used.

The results show that it is very easy to achieve a spot capacity between 10 and 20, but it is extremely difficult to reach 25 and practically impossible to exceed 30 except in very favourable circumstances. The spot capacity increases with decreasing diffusion coefficient and increasing kinetic coefficient of the solvent and plate quality (packing homogeneity). For a given solvent and development length there is an optimum particle size that provides the maximum spot capacity. The fastest separation is achieved using slightly larger particles. The spot capacity also increases with increasing development distance towards a limit.

INTRODUCTION

The concept of peak capacity is probably the simplest and most straightforward for qualifying and comparing the resolution power of a chromatographic system. The peak capacity is the maximum number of components of a mixture that can be resolved with a resolution of unity between the inert peak and the most retained solute[1]. This is obviously the maximum number of compounds that can be separated with the system studied. In fact, as it is impossible to space regularly all compounds on the chromatogram, the peak capacity of the system used should always greatly exceed the number of compounds one wants to resolve.

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Giddings has given mathematical expressions for the peak capacity, n, of chromatographic separations[1] and for other separation methods[2]. A simple and excellent approximation has been derived by Grushka[3], assuming that the number of theoretical plates corresponding to the various peaks with different retentions is constant and the same for all solutes. Although this is not exact, and far from true for open-tubular columns[4,5], it is an excellent approximation for packed columns, for which the plate number rarely varies by more than 10–20% along the chromatogram, except perhaps for some compounds with unusual properties (polarity, reactivity, etc.).

The equation derived by Grushka[3] is

$$n = 1 + \frac{\sqrt{N}}{4} \cdot \ln (1 + k') \tag{1}$$

where n is the peak capacity, N the plate number and k' the column capacity factor for the most retained compound, the last to be recorded before the analysis is considered to be finished.

This value of k' can be chosen in different ways. For example, as dilution is an essential part of the chromatographic process, and sample size is limited by the non-linearity of isotherms, there is a finite value of the retention, beyond which the concentration at peak maximum will be smaller than the detection limit[6–8]. Alternatively, Guiochon[9] has shown that in time normalization (constant analysis time), the maximum peak capacity is reached for $k' \approx 6.4$ [then $\ln (1 + k') = 2$]. If k' is larger it would be possible to increase n by using a longer column (larger N) and recording the chromatogram for a shorter range of k', using a slightly stronger eluent, and conversely if k' is smaller than 6.4 we could increase n by using a shorter column and a larger range of k' with a weaker solvent[9].

Accordingly, the theoretical peak capacity of a chromatographic column is

$$n_t = 1 + \frac{\sqrt{N}}{2} \tag{2}$$

the corresponding analysis time being $7.4t_m$. A survey of the analytical literature shows that most published chromatograms cover a range of k' between 0–5 and 0–10, which is in agreement with this result.

It is much less straightforward to estimate the peak capacity in thin-layer chromatography (TLC), as the plate number cannot be considered as constant in this mode of chromatography. It is easy to show that the HETP is a function of the development length, of the retention and of many conventional parameters of the system[10–12].

THEORETICAL

The peak or spot capacity in TLC can be found from the fact that the number of spots with a resolution of unity on a short length of plate, dz, is given by[3]

$$dn = \frac{dz}{4\sigma} \tag{3}$$

where z is the distance along the plate and 4σ is the width of the spot; this equation is easily derived in the same way as the similar equation for column peak capacity[3].

In some instances, when very small particles are used to make the thin layer and excessive development length and time are used, the spot width becomes fairly constant, almost independent of the solute[11]. This width can be derived from the spot variance:

$$\sigma^2 = \sigma_i^2 + 2\gamma Dt \tag{4}$$

where $4\sigma_i$ is the width of the sample zone as deposited on the TLC plate, D the diffusion coefficient of the solute, assumed to be the same for all solutes, which is almost equivalent to assuming a constant plate number in column chromatography, t is the time and γ the tortuosity of the packing. Although γ is usually assumed to be constant, we explain below that there are reasons to think it is not so but varies to some extent with retention[13] and mobile phase velocity[14]. The analysis time is related to the development length by the classical quadratic law

$$L^2 = kt \tag{5}$$

where k is a kinetic constant, related to the experimental conditions by

$$k = -2k_0 d_p \cdot \frac{\gamma'}{\eta} \cdot \cos\Theta = -\theta \cos\Theta d_p \tag{6}$$

where d_p is the particle average diameter, γ' the surface tension of the solvent, η its viscosity and θ its contact angle on the material used to make the particles[12] and k_0 is a constant which is approximately equal to $8 \cdot 10^{-5}$. In normal-phase chromatography the adsorbent is completely wetted by all solvents and $\cos\Theta = -1$. This is not valid in reversed-phase (RP) chromatography where water–organic solvent mixtures do not wet totally the non-polar chemically bonded phases[15]. Development is slower than the consideration of Θ alone would predict and may even be impossible if $\Theta < 90°$. Combination of eqns. 4 and 5 gives a value of the spot width that is independent of the retention or position of the spot on the plate. Combination with eqns. 3 and 4 and integration gives

$$n = \frac{L}{4\sqrt{\sigma_i^2 + \dfrac{2\gamma DL^2}{k}}} \tag{7}$$

as for given development time and plate characteristics, L is a constant. In still too many practical cases, σ_i is large compared to the other term and n is determined by the sampling ($n \approx L/4\sigma_i$) and is rather small.

When L increases, n becomes larger until it reaches a limit, when the spot width is large compared with the diameter of the original sample spot, $4\sigma_i$ (ref. 11). Then

$$n_T = \sqrt{\frac{k}{32\gamma D}} \tag{8}$$

is independent of the development length. This is because a quadratic law controls both diffusion and solvent migration in TLC and accordingly the spots spread at the same speed that they move away from each other[11].

Eqn. 8 is only approximate, however, not only because it is valid only with long development times (so σ_i becomes negligible) but also because it assumes that the plate number is controlled only by axial diffusion and by diffusion in the mobile phase. When TLC is properly developed the mobile phase velocity is larger than or around the optimum velocity during a significant fraction of the analysis time[11]. Accordingly, we must take into account the variation of the plate number with development length and with retention. The spot variance is given by

$$\sigma^2 = \sigma_i^2 + zH \tag{9}$$

where

$$z = L_f/R_F \tag{10}$$

is the migration distance of a spot and H is the height equivalent to a theoretical plate corresponding to that spot. H is related to the experimental conditions by the conventional equation[10]

$$H = b(L + z_0) + \frac{a}{L - z_0}(L^{2/3} - z_0^{2/3}) + \frac{c}{L - z_0} \cdot \ln\left(\frac{L}{z_0}\right) \tag{11}$$

where the coefficients a, b and c are related to the coefficients of the plate-height equation:

$$a = \frac{3Ad_p^{5/3}\theta^{1/3}}{2(2D_m)^{1/3}} \tag{12}$$

$$b = \frac{B}{\theta d_p} \tag{13}$$

$$c = \frac{C\theta d_p^3}{2D_m} \tag{14}$$

Eqn. 11 has been derived by integration[10], using the quadratic law (eqn. 5) for the flow velocity and assuming that the plate height is related to the mobile phase velocity by the Knox reduced plate height equation[16,17]:

$$h = \frac{B}{v} + Av^{1/3} + Cv \tag{15}$$

The dimensionless coefficients A, B and C characterize the packing homogeneity and the flow stream pattern around the particles (A), axial diffusion (B) and the resistances to mass-transfer inside the particles (C). A is observed to be reasonably con-

stant for a given column, independent of the solute and mobile phase; it depends on the packing technology and can be as low as 1 in column chromatography and 0.6–0.7 in TLC[10]. C depends on the R_F value in a complex way[18,19]. As a first approximation it will be assumed that it is constant, which is not exact for small retentions (R_F values close to 1); for small particle sizes as currently used in TLC (below *ca.* 20 μm), however, the third term in eqn. 10 is very small, often even negligible, so the assumption that C to be constant introduces a small error in the following calculations. The first term of eqn. 10 tends to be the most important, especially with long development lengths and, unfortunately, it is the least well known. If one takes into account the contribution of axial diffusion in the stationary phase, the B term has been expended[12] as

$$B = 2\left(\gamma_m D_m + \frac{1 - R_F}{R_F} \cdot \gamma_s D_s\right) \tag{16}$$

where γ is the tortuosity factor, D the diffusion coefficient and the subscripts m and s represent the mobile and stationary phase, respectively. B, and accordingly H with long development lengths, is inversely proportional to R_F if $\gamma_m D_m \approx \gamma_s D_s$. Thus H is infinity for $R_F = 0$, which is normal as the spot has not moved but has acquired a finite diameter, through diffusion. On the other hand, if $\gamma_s D_s$ is negligible, B is independent of R_F. This seems to be a rare situation and in the following we have assumed that $\gamma_m D_m$ is equivalent to $\gamma_s D_s$, which is the worst situation as it predicts the lowest level of performance.

When B is independent of R_F, so is H (*cf.*, eqn. 11) as we may assume that c is either negligible or independent of R_F as the third term of the right-hand side of eqn. 11 is always small compared with the other two. If H is constant we can combine eqns. 3 and 9:

$$dn = \frac{dz}{4\sqrt{\sigma_i^2 + Hz}} \tag{17}$$

which is integrated between $z = 0$ and $z = L$ to give

$$n = \frac{\sqrt{\sigma_i^2 + LH} - \sqrt{\sigma_i^2}}{2H} \tag{18}$$

where H is given by eqn. 11. n increases with decreasing sample spot diameter and its maximum value is

$$n_M = \frac{1}{2}\sqrt{\frac{L}{H}} \tag{19}$$

If H is not independent of R_F it becomes impossible to calculate n explicitly. A numerical calculation can be made, however, assuming that the distance between two successive spots, p and $p + 1$, separated with a resolution of unity, is equal to the diameter of spot p. The migration distances of spots p and $p + 1$ are thus related by:

$$z_{p+1} = z_p + 4\sigma_p \tag{20}$$

where σ_p is a function of z_p. The spot capacity, n, is calculated such that

$$\sum_{p=0}^{n} 4\sigma_p \leqslant L - z_0 < \sum_{p=0}^{n-1} 4\sigma_p \tag{21}$$

The calculation is easily made using an HP 67 calculator, following the scheme detailed in Fig. 1.

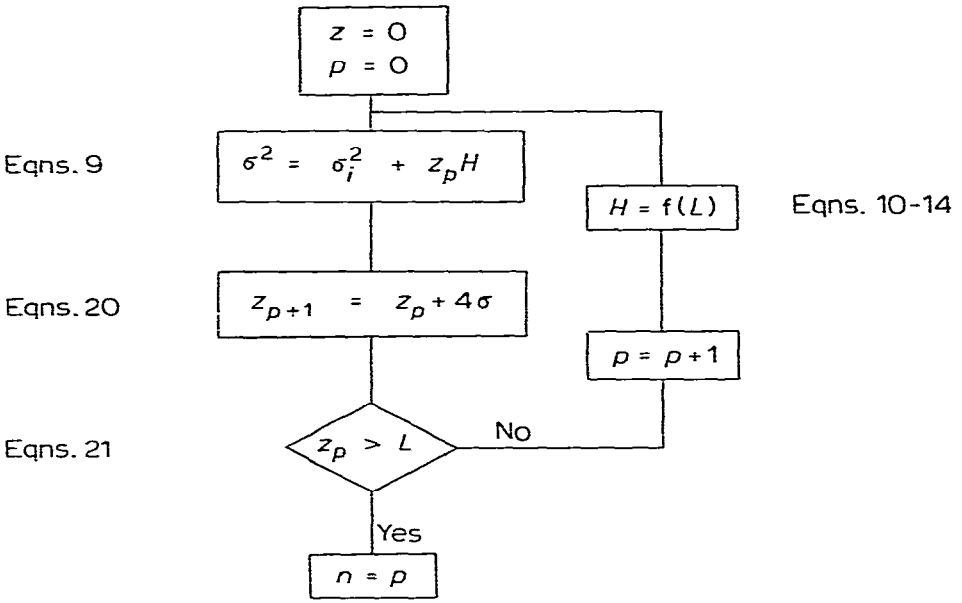

Fig. 1. Computation of the spot capacity in TLC (eqns. 9, 20 and 21).

Finally, we wish to emphasize that we just want to calculate here the total number of spots that can be placed on a plate, each being separated with a resolution of unity from its neighbours. Therefore, the relative retention $R_{F,p+1}/R_{F,p}$ of two successive such spots is not constant but is a minimum for some intermediate value of R_F, depending on the relationship between σ_p and R_F (ref. 20).

EXPERIMENTAL

Spots of solutions of triazene derivatives were placed on various Merck (Darmstadt, G.F.R.) TLC plates (plain silica or RP-18), made with 5-μm particles, using either a 7001 Hamilton syringe with a straight tip needle or a very fine glass capillary. These plates were scanned either immediately or after development over various lengths, using a Zeiss spectrophotodensitometer. The shortest time constant available was selected.

The compounds used were bisphenyltriazene, bis(4-chlorophenyl)triazene,

bis(2,4-) and bis(2,5-dichlorophenyl)triazene and bis(3-cyanophenyl)triazene, prepared in our laboratory, and Red and Yellow Desaga dyes.

Developments were carried out in an N chamber (Camag, Muttens, Switzerland) with methanol (RP-18 plates) or benzene (silica plates).

RESULTS AND DISCUSSION

The spot capacity of TLC was calculated for different combinations of development length and particle size, and also using values of the parameters typical of many different systems, *i.e.*, using solvents with different kinetic parameters and solutes with different diffusion coefficients. The results are presented in Figs. 2–4 and Tables I–VI.

Except for alcohols heavier than methanol, for water–organic solvent mixtures and for non-conventional solvents, k/d_p is between 60 and 120 cm/sec, γ is *ca.* 0.7 and for most systems used in TLC, D is between $5 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ cm/sec but could be smaller for large molecules analysed in biochemistry.

Thus the extreme limits of the range of spot capacities that can be achieved in principle, as calculated from eqn. 7, are given in Table I. No sample of 2-μm silica is available yet. Depending on the conditions, the spot capacity ranges between 7 and 46, the lower value being obtained for the smaller particle sizes as usual in chromatography[9]. Eqn. 2 shows that to reach a peak capacity of 46 a column must provide 8200 theoretical plates, which is easily achieved with current column technology. In fact, as will be seen below, it is extremely difficult in practice to achieve a spot capacity of more than 30.

TABLE I

SPOT CAPACITY IN TLC ($\gamma = 0.7$) (EQN. 8)

Parameter	$\theta = k/d_p$ *(cm/sec)*			
	60	*120*	*120*	*60*
D (cm^2/sec)	$1 \cdot 10^{-5}$	$5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
d_p (μm)	2	2	20	20
n	7	14	46	23

Other results derived from previous efficiency calculations[12] are reported in Table II, obtained using eqn. 19. They demonstrate that the use of small particles is not necessary in order to achieve large spot capacities, rather the opposite. Further, as we show later, the influence of sample spot size is much smaller for long than for short developments.

Two sources of problems limit in practice the spot capacity that can be achieved in TLC: the diameter of the original spot and the development time.

Influence of sample size

When a spot of a solution is placed on a TLC plate, the solution tends to penetrate inside the particles by capillarity, and very little remains between the par-

TABLE II

THEORETICAL SPOT CAPACITY IN TLC (EQN. 19)

Diffusion coefficient: $5 \cdot 10^{-6}$ cm^2/sec. HETP data from ref. 12, Fig. 6. θ = 47.2 cm/sec (*n*-octane, cyclohexane, carbon tetrachloride, dioxane). $\sigma_i = 0$.

Parameter	*Plate length, L (cm)*					
	2	*5*	*5*	*10*	*10*	*10*
Particle size, d_p (μm)	5	5	10	5	10	20
HETP (μm)	18	25	22	47	30	50
n_M	16	22	23	23	28	22
t (min)	2.8	17.7	8.8	70.6	35.3	17.7

ticles, assuming that nothing is lost by vaporization during this process, which is very rapid. The diameter of the sample spot, d_s, is given by the equation

$$V_s = \frac{\pi d_s^2}{4} \cdot e\varepsilon_i (1 - \varepsilon_e) \tag{22}$$

which assumes a circular distribution where all particles inside the spot are filled with solution and none of the solution is outside this circle. V_s is the sample volume, ε_e the external porosity of the packing and ε_i the particle porosity. Typically e is approximately 0.25 mm, although some workers use thinner plates, especially when they are using small plates made with fine particles. The external porosity is always *ca.* 0.4 for a dense packing and ε_i is 0.4–0.5 for most silica packings used in normal and RP chromatography. Then d_s is *ca.* 4.3 mm for a 1-μl sample (1 mm^3). Such a sample is injected into the chromatographic system as a plug, however, and according to Sternberg[21] the corresponding standard deviation is given by

$$\sigma_i^2 = \frac{d_s^2}{12} = \frac{V_s}{3\pi e\varepsilon_i (1 - \varepsilon_e)} \tag{23}$$

The value is 1.25 mm in this instance, which is very large. Samples much smaller than 1 μl must be used in TLC, unless zone concentration techniques are used[22].

Experimental results show that eqn. 23 gives only the order of magnitude of the sample spot standard deviation. With silica and chemically bonded silica, ε_i is typically *ca.* 0.65, although this value may change markedly from batch to batch. ε_e is always between 0.38 and 0.40. Accordingly, we can assume that $\varepsilon_i(1 - \varepsilon_e)$ is close to 0.40, a value in agreement with that used by Fenimore[23]. With V_s = 0.1 μl, the "apparent" spot diameter when using a strongly eluting solvent is in agreement with the result of eqn. 22. On the other hand, eqn. 23 predicts σ_i = 0.33 mm whereas the experimentally measured value using the Hamilton syringe is 0.58 mm, which would be in agreement with a more conservative estimate:

$$\sigma_i = \frac{d_s}{2} \tag{24}$$

The standard deviation can be reduced further by using a smaller sample and a fine capillary (we could then achieve σ_i between 0.1 and 0.2 mm) or depositing the sample under a stream of nitrogen or air to accelerate vaporization of the solvent and by using, if possible, a weak solvent. Using this last technique, Fenimore[23] was able to reduce d_s from 1.4 mm for a 0.2-μl sample, in agreement with the value predicted by eqn. 22, to 0.45 mm by replacing acetone with *n*-heptane. We also observed that the use of a weak solvent results in a steeper sample profile[24], thus reducing further the standard deviation, which for a steep profile becomes intermediate between $d_s/2$ (eqn. 24) and $d_s/\sqrt{12}$ (eqn. 23). Using a specially designed instrument, Fenimore[23] was able to achieve routinely sample spots between 0.13 and 0.45 mm in diameter with apparently steep profiles, corresponding to standard deviations between 0.05 and 0.20 mm. Using more conventional instrumentation we have obtained standard deviations between 0.3 and 0.6 mm depending on the experimental conditions.

An investigation of the effect of the standard deviation of the introduced sample has been made by calculating the peak capacity as a function of this standard deviation in a few typical examples (*cf.*, Fig. 2 and Table III). In many instances only a moderate fraction of the maximum spot capacity predicted by eqn. 8 is achieved. It is seen that when keeping everything else constant but increasing the development length, the spot capacity tends towards a maximum but the analysis time increases rapidly. Except for very short development lengths (< 5 cm) and for low values of the

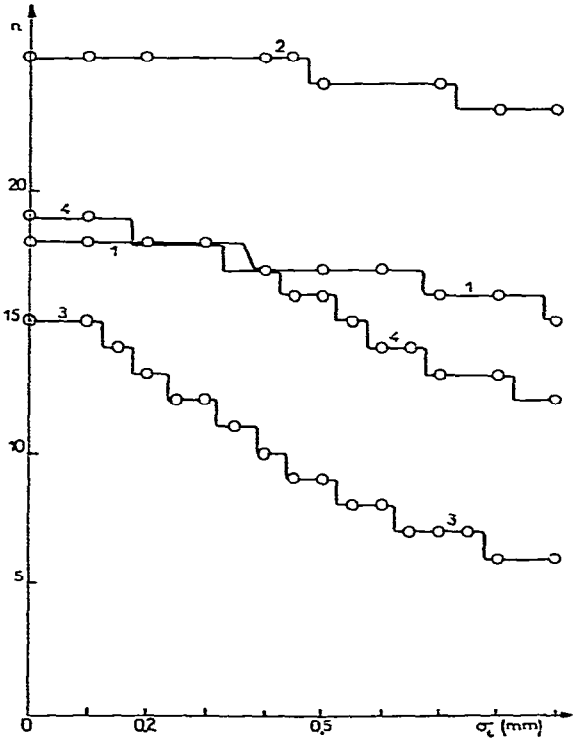

Fig. 2. Variation of spot capacity with the standard deviation of the sample spot. Conditions for curves 1–4 in Table III.

TABLE III

INFLUENCE OF SAMPLE SIZE ON SPOT CAPACITY

$\gamma = 0.70$; $A = 1$; $C = 0.01$.

Parameter	*Curve No. (Fig. 3)**									
	1	*NS*	*NS*	*NS*	*2*	*3*	*4*	*NS*	*NS*	*NS*
D_m (cm^2/sec) · 10^6	5	5	5	5	5	5	5	*1*	1	1
θ (cm/sec)	47	*120*	120	120	120	120	120	120	120	120
d_p (μm)	10	10	10	*20*	20	*5*	5	5	*2*	2
L (cm)	10	10	*5*	*10*	*20*	*2*	5	5	*2*	*5*
z_0 (cm)	0.5	0.5	0.5	0.5	0.5	*0.2*	*0.5*	0.5	*0.2*	*0.5*
n_M (eqn. 8)	20	32	32	46	46	23	23	51	32	32
n_0 ($\sigma_i = 0$)	18	23	18	19	25	15	19	26	23	28
t (min)	35	14	3.5	7	28	1.1	7	7	3	17
$\sigma_{i,max(mm)}$	0.85	0.55	0.40	0.65	0.95	0.15	0.40	0.20	0.10	0.25

* NS: curve not shown in Fig. 3 for sake of clarity. The figures in italics are those which differ from the figures in the previous column.

** $\sigma_{i,max}$ is the standard deviation of the sample spot for which the spot capacity is reduced by 10%.

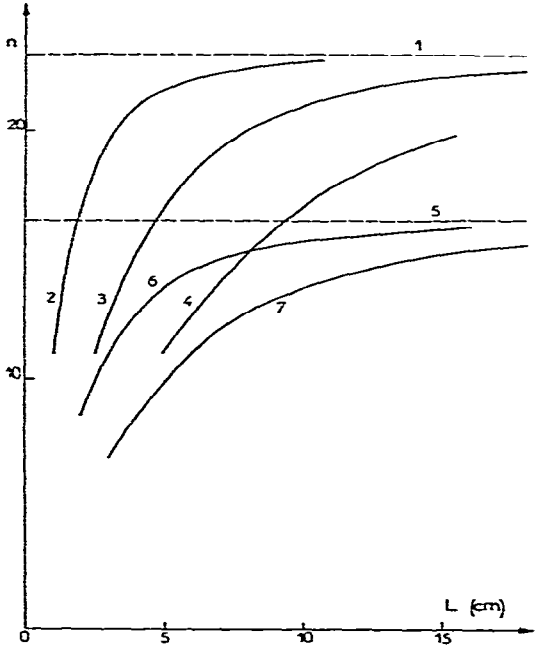

Fig. 3. Variation of spot capacity as predicted by eqn. 7 (diffusion-controlled spot diameter) as a function of column length for various sample sizes. In this instance the spots are circular. $D = 5 \cdot 10^{-6}$ cm^2/sec, $d_p = 5$ μm, $\theta = 120$ cm/sec, $\gamma = 0.7$: curve 1, $\sigma_i = 0$; 2, $\sigma_i = 0.2$; 3, $\sigma_i = 0.5$; 4, $\sigma_i = 1$ mm. $D = 5 \cdot 10^{-6}$ cm^2/sec, $d_p = 5$ μm, $\theta = 60$ cm/sec, $\gamma = 0.7$: curve 5, $\sigma_i = 0$; 6, $\sigma_i = 0.5$; 7, $\sigma_i = 1$ mm. Development time for 10 cm: 28 min for $\theta = 120$ and 56 min for $\theta = 60$.

diffusion coefficient, the maximum acceptable sample standard deviation, defined here as that resulting in a reduction of the spot capacity by 10%, is *ca.* 0.4–0.6 mm, corresponding to a sample volume of about 0.1 μl, as discussed above, which is small but still acceptable and corresponds to current practice. Fig. 3 shows the variation of the spot capacity with development length as predicted by eqn. 7 for different values of the sample spot standard deviation and two different sets of conditions. With small development lengths, the spot capacity depends mainly on the sample size. The use of short development lengths requires extremely small sample spots. In the following only the spot capacity at zero sample size will be calculated in most instances.

Influence of development length and particle size

In most instances, when large spot capacities are necessary, it is more effective to change the experimental conditions, and especially to increase the particle size and development time, rather than merely to increase the development length alone, but the situation is complex and deserves detailed discussion. The spot capacity is given in Table IV for combinations of development lengths between 1 and 30 cm and particle sizes between 2 and 25 μm. The analysis time increases in proportion to the square of the development length (on each line in the Table IV) and decreases in proportion to the inverse of the particle size (along each column). The spot capacity increases monotonically with increasing development length, but although it is easy to achieve values close to the theoretical maximum (eqn. 8) with small particles, it becomes prohibitively difficult with large particles, with which very large development lengths and analysis times would be necessary. It is almost impossible to carry out TLC in practice with the large development chambers (1-m long) and the long time necessary (more than 9 h for a 1-m long plate made with 25-μm particles) to achieve a spot capacity of 41, instead of a theoretical maximum of 52 with this particle size.

TABLE IV

INFLUENCE OF DEVELOPMENT LENGTH AND PARTICLE SIZE ON SPOT CAPACITY

$A = 1$; $C = 0.01$; $\gamma = 0.7$; $D_m = 5 \cdot 10^{-6}$ cm²/sec; $\theta = 120$ cm/sec. The development time varies from 41 sec ($L = 1$, $d_p = 2$) to 3 sec ($L = 1$, $d_p = 25$) and from 50 min ($L = 30$, $d_p = 25$) to 178 min ($L = 30$, $d_p = 7$).

d_p (μm)	ΔL^* (cm)						n_M	Performance for $n = n_M/2$	
	1	2	5	10	20	30		ΔL^* (cm)	t_A
2	13**	14**	15				15	–	–
3	14**	16	17	18			18	–	–
5	13**	16	20	21	23		23	0.9	14 sec
7	12**	15	20	23	25	26	27	1.3	20 sec
10	10	13	19	24	28	29	33	3	75 sec
15	8	11	16	22	27	31	40	8	6 min
20	7	9	14	19	26	30	46	14	13.6 min
25	6	8	12	17	23	28	52	25	35 min

* $\Delta L = L - z_0$, *i.e.*, the theoretical length of migration of a non-retained compound. $z_0 = 0.2$ cm for $L < 5$ cm; $z_0 = 0.5$ cm for larger L.

** $\sigma_{i,max} \approx 0.15$ mm or smaller.

With a constant development length the spot capacity passes through a maximum for a given particle size. For smaller particle sizes, zone spreading by molecular (axial) diffusion due to too slow a development prevails. The spots are almost circular and their length increases only very slowly with increasing R_F. With larger particles, inhomogeneous flow patterns between the particles becomes the dominant source of band broadening; the spots are long (in the development direction) and narrow and their length increases rapidly with increasing R_F. Their width in the direction perpendicular to the development is given by eqn. 4. These differences are illustrated in Fig. 4, which illustrates chromatograms having a spot capacity of 12–13, but obtained under different conditions, as given in Table IV. The abscissa is R_F, so the lengths of the actual chromatograms are very different (*cf.*, Fig. 4).

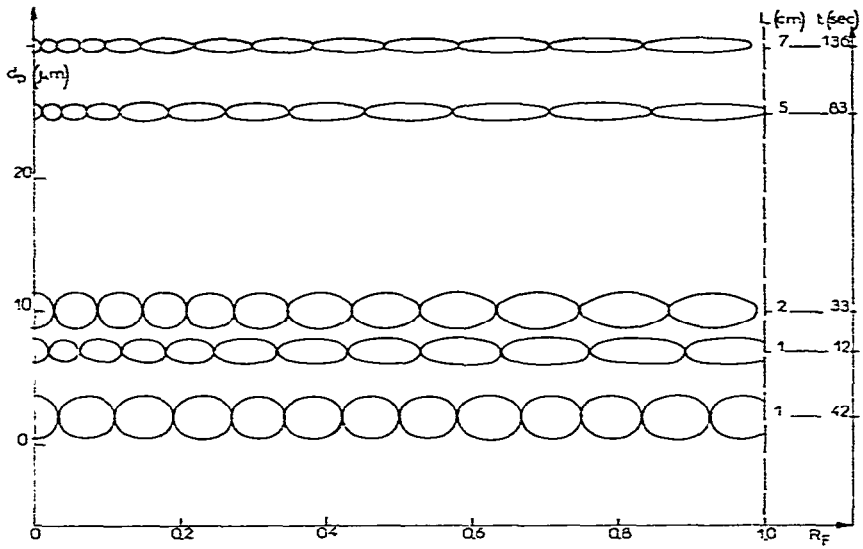

Fig. 4. Thin-layer chromatograms exhibiting similar spot capacity (12–13) obtained under various conditions (*cf.*, Table IV). Particle size, left-hand ordinate, development length and time, right-hand ordinate. The chromatograms have all been normalized, using R_F as common unit. For $d_p = 2$ μm molecular diffusion is the main source of band broadening while eddy diffusion and mass transfer predominate for 25 and 30 μm.

The resolving power of small plates made with very fine particles is very high and a 1-cm long chromatogram has a spot capacity as high as 13. Such performances are difficult to use in practice for real measurements. Besides the sampling problem already discussed, which in this instance will impose drastic requirements on sample size, the technical problems of scanning such a chromatogram with high accuracy have not yet been solved[25].

Another conclusion from these data is the extreme narrowness of the range of performances that can be achieved in TLC. Under the conditions selected for the previous calculations, it is very easy to achieve a spot capacity of 15, difficult but possible to achieve 20–25 and practically impossible to exceed 30. The analysis time is less than 1 min in the first instance ($n = 15$; $L = 2$; $d_p = 7$ μm) and slightly exceeds 1 h in the last ($n = 30$; $L = 30$; $d_p = 20$ μm). As a matter of comparison the corre-

sponding peak capacities are achieved with columns producing 800 and 3400 theoretical plates, respectively. With classical packed columns made with 5-μm particles having a moderately reduced efficiency of 3, this last performance could in theory be achieved with a 5-cm long column. At a reduced velocity of 3 the analysis time ($k' = 6.4$) would be 21 min with an inlet pressure for a solvent with a viscosity of 1 cP of 6 atm. This is still a very simple analysis and most current LC analyses are carried out at a better performance level.

It has been found that the influence of z_0 is negligible if it is smaller than $0.1L$. As it is not an important parameter in practical TLC, no further discussion is necessary (*cf.*, Table III).

Influence of solvent characteristics

The choice of the solvent determines the kinetic parameter θ and the diffusion coefficient of the solute, although the values of these factors are rarely taken into account when selecting a solvent for TLC. Calculations have been made for different values of the diffusion coefficient and kinetic parameters. They are reported in Table V.

The diffusion coefficient is varied between $1 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ cm^2/sec, which covers all practical cases in TLC except for large molecules (molecular weight several thousand Daltons or more). For each combination of development length and par-

TABLE V

INFLUENCE OF KINETIC PARAMETER AND DIFFUSION COEFFICIENT ON THE SPOT CAPACITY

$A = 1$; $C = 0.01$; $\gamma = 0.7$.

Dm** (cm^2/sec)	ΔL* (cm)							
	2		5		10			20
	d_p (μm)							
	2	5	5	10	5	10	20	10
$1 \cdot 10^{-6}$	24	18	26	18	34	27	17	36
$2 \cdot 10^{-6}$	20	18	24	19	29	26	19	34
$5 \cdot 10^{-6}$	14	16	20	19	21	24	19	28
$1 \cdot 10^{-5}$	10	14	15	17	16	20	19	22
θ*** (cm/sec)								
40	9	12	13	15	13	17	18	18
60	10	14	15	17	16	20	19	22
80	12	15	17	18	18	21	19	24
100	13	15	19	18	19	23	19	26
120	14	16	20	19	21	24	19	28

* $\Delta L = L - z_0$; $z_0 = 0.2$ cm for $L < 5$ cm; $z_0 = 0.5$ cm for larger L.
** $\theta = 120$ cm/sec.
*** $D_m = 5 \cdot 10^{-6}$ cm^2/sec.

ticle size, there is an optimum value of the diffusion coefficient. When molecular diffusion is controlling the spot broadening, clearly the spot capacity increases with decreasing diffusion coefficient, as the spots become narrower. There is an optimum, however, because the coefficients of the second and third terms of the plate-height equation increase with decreasing diffusion coefficient. This is because the actual mobile phase velocity profile depends only on the plate length and particle size, but when the diffusion coefficient decreases, the corresponding reduced velocity increases, and eventually the flow unevenness and the resistance to mass transfer become the main source of band broadening. Unless the particle size is very large, however, better spot capacities are obtained with the small values of the diffusion coefficient in the range studied, which is also in agreement with the monotonic increase in the theoretical limit of the peak capacity with decreasing diffusion coefficient (eqn. 8).

The kinetic coefficient of the solvent is varied between 40 and 120 cm/sec, which again covers all practical cases in TLC. Very few solvents have a kinetic coefficient outside these limits[12]. The only notable ones are carbon disulphide and diisopropyl ether (*ca.* 140), ethanol ($\theta = 32$) and heavier alcohols, and a few exotic solvents, never used because they are too viscous, although the development of small, fast plates could make them more attractive. The data in Table V, however, show that the spot capacity increases steadily with increasing kinetic coefficient, as well as the theoretical limit of n (eqn. 8), except with large particle sizes, where it is almost constant. Although the variation is slow, the increase in peak capacity exceeds one third to one half over the entire range of kinetic coefficients.

The combination of a small plate ($\Delta L = 2$ cm) and coarse particles (20 μm) permits the achievement of a spot capacity of 9 for $\theta = 20$, with a development time of less than 2 min. This is still an acceptable performance in a situation bad enough to require aniline ($\theta = 16$) or formamide ($\theta = 25$) as the mobile phase, or organic solvent–water mixtures with a low wetting angle and values of $\cos\theta$ larger than -0.5 (ref. 15).

Influence of the plate characteristics

Very little is known about the important parameters which are the coefficients of the reduced plate-height equation. In a recent paper Thumneum and Hawkes[14] demonstrated what had been suspected for a long time, that the axial diffusion term is not well understood and that the tortuosity coefficient of the packing is not constant, but varies with the mobile phase velocity. Although the experimental data have been obtained only in gas chromatography, there is only one reason to think the situation can be different in liquid chromatography: GC is carried out normally in a range of Reynolds numbers much larger than in LC and the variation of γ with the velocity is probably related to the dynamics of the fluid mobile phase. In both instances the Reynolds number are smaller than 1: *ca.* 0.3 for helium at 10 cm/sec through a 150-μm particle size column[14] and *ca.* $3 \cdot 10^{-3}$ for an organic solvent at a reduced velocity of 3 through a 10-μm particle size column. This problem certainly deserves careful study, especially in TLC where the corresponding term is often the major contribution to band broadening. In column chromatography A is typically between 1 and 3. An A value of 1 corresponds to an excellent column (optimum reduced HETP $\approx$ 2) and it is extremely difficult to pack a better column, if at all possible, at least on a routine basis. It seems that making a homogeneous thin bed is easier than packing a

column; the segregation of particles of different sizes cannot take place at such a large distance. The technique used to spread the particle slurry permits good lateral homogeneity, *i.e.*, the solvent front is parallel to the edge of the plate and to the solvent level in the tank. Segregation of particles in the slurry during the preparation of the plates will mainly result in a systematic variation of particle size along the plate, *i.e.*, in a systematic change of the kinetic parameters along the migration distance and to deviation from the quadratic law. This affects more the reproducibility of the retention data than the resolving power. In all instances where attempts have been made to fit an HETP equation to experimental data[10,24] values of A smaller than 1 have been found. In spite of the number of adjustable constants, no satisfactory fit could ever be obtained with values of A larger than 1.

Typical values of C in liquid chromatography are found[16,17] to be between 0.01 and 0.1; they are the same in TLC and column chromatography.

Accordingly, calculations have been carried out for values of A between 0.5 and 2 and values of C between 0.01 and 0.1. As shown in Table VI, the effect of C is minor provided that it is smaller than 0.1, which is the case for most packings used in liquid chromatography. The effect of C is smaller in TLC than in column LC. Although larger, the effect of changing A on the spot capacity remains moderate, except when packing heterogeneity controls band broadening, which is the case when short plates are made with large particles and developed with a fast solvent.

Comparison with the results of the optimization approach

In a previous paper[12] we developed a model of band migration and band

TABLE VI

INFLUENCE OF THE CHARACTERISTICS OF THE CHROMATOGRAPHIC BED ON THE SPOT CAPACITY

$\gamma = 0.7$; $\theta = 120$ cm/sec; $D_m = 5 \cdot 10^{-6}$ cm²/sec.

*A***	*ΔL** (cm)							
	2		*5*		*10*			*20*
	d_p (μm)							
	2	*5*	*5*	*10*	*5*	*10*	*20*	*10*
0.5	14	18	21	22	22	27	24	30
0.7	14	17	20	20	22	25	21	29
1.0	14	16	20	19	21	24	19	28
2.0	13	14	18	15	20	20	15	25
*C****								
0.01	14	16	20	19	21	24	19	28
0.05	14	16	20	18	21	23	18	27
0.10	14	15	19	17	21	23	17	27

* $\Delta L = L - z_0$; $z_0 = 0.2$ cm for $L < 5$ cm; $z_0 = 0.5$ cm for larger L.
** $C = 0.01$.
*** $A = 1$.

TABLE VII

MAXIMUM PLATE NUMBER ACHIEVED FOR A GIVEN TIME AND SPOT CAPACITY

$A = 1$; $C = 0.01$; $\gamma = 0.75$; $z_0 = 0.5$ cm for $L > 5$ cm; $D = 5 \cdot 10^{-6}$ cm^2/sec; $\theta = 50$ cm/sec.

t (sec)	N_{max}	d_p (μm)	L (cm)	$\sqrt{N_{max}}/2$	n_T	
					$\sigma_i = 0$	$\sigma_i = 0.05$ cm
20,000	2850	34	58	27	28	28
10,000	2260	27	37	24	25	25
5000	1795	21.5	23	21	22	22
2000	1320	15.9	12.5	18	19	18
1000	1050	12.6	8.	16	17	16
500	833	10.0	5.	14	15	13
200	615	7.4	2.7	12	13	9

spreading in TLC and applied it to the optimization of experimental conditions in this technique, with the aim of indicating to the analyst which circumstances to avoid and which ones to prefer.

It therefore appears interesting to calculate the spot capacity for a series of optimum conditions and to compare the values obtained with the number of theoretical plates achieved. The results of this calculation are given in Table VII. The other data in this Table are taken from our previous paper[12]. It appears that provided the size of the sample spot is neglected,

$$n = 1 + \frac{\sqrt{N_{max}}}{2}$$

which is in agreement with the similar relationship obtained in column chromatography. As the values of n and N_{max} are obtained from a completely different calculation, this agreement illustrates the self-consistency of our model. The decrease in spot capacity resulting from the sample spot size is significant for short developments, as discussed above.

This again illustrates the difficulties of TLC. A 100-fold increase in analysis time allows the achievement of four times more theoretical plates and doubles the spot capacity. This is a poor result considering the price paid in terms of analysis time. In practice, the analyst is faced with a "take it or leave it" situation: if the separation is not achieved on a given plate he has to look for another chromatographic system. At most he could check that the combination of development length and particle size he is using ensures that the solvent flow-rate is neither too fast (this is rare) nor too slow (this is too frequent), and that the experimental conditions are not too far from the optimum[12].

Comparison with experimental data

Fig. 5 shows plots of spot variance (σ^2) derived from the chromatograms obtained with the spectrophotodensitometer *versus* the square of the development length. The spot variance in the direction perpendicular to the development increases with increasing development length, as predicted by eqn. 4, with $t = L^2/\theta d_p$, while the

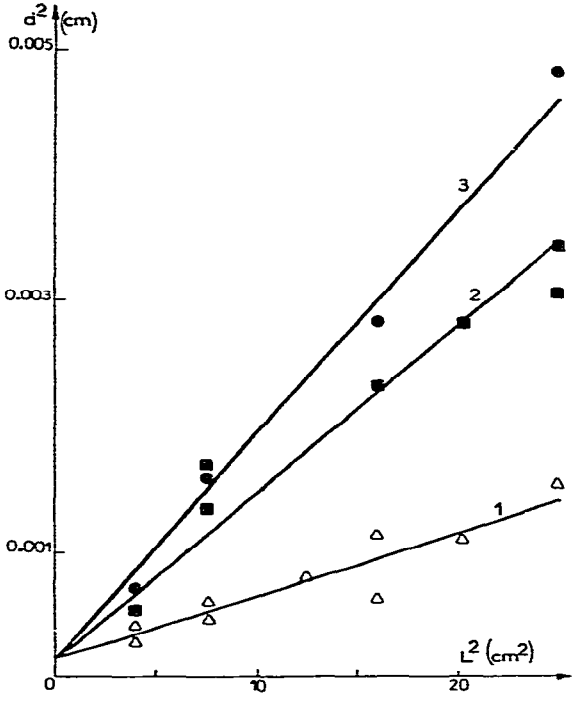

Fig. 5. Plot of spot variance *versus* square of development length using silica (5 μm) and benzene. Solutes: Red Desaga, R_F = 0.31, and Yellow Desaga, R_F = 0.66. Line 1: red spots, variance in the direction perpendicular to development. $D = 1.3 \cdot 10^{-6}$ cm²/sec. Line 2: red spots, variance parallel to the development. H = 21 μm (theoretical calculation, 17 μm). Line 3: yellow spots, variance parallel to the development. H = 14 μm (theoretical calculation, 12.5 μm). All three lines give σ_i = 0.12 mm.

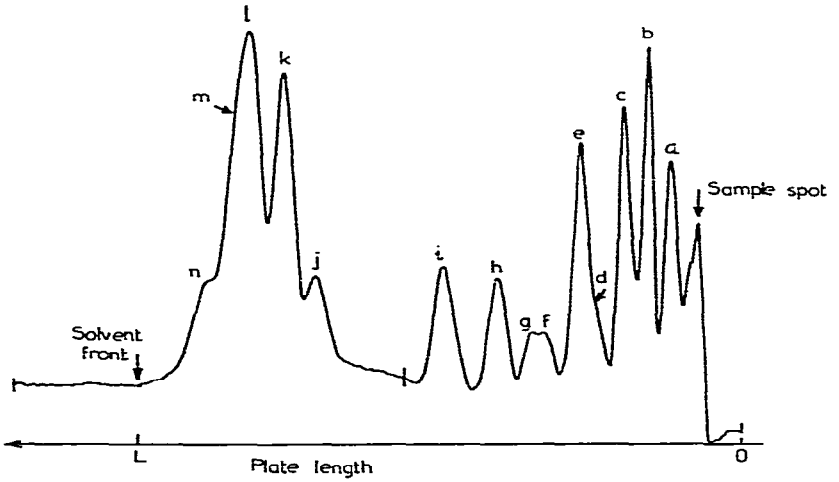

Fig. 6. Separation of a complex mixture on a HPTLC plate (data Table VIII, line 3). a = Bis(3-hydroxymethylphenyl)triazene; b = bis(2-pyridoxyphenyl)triazene; c = bis(2-cyanophenyl)triazene; d = bis(carbethoxyphenyl)triazene; e = choro 4-phenyl, phenyl triazene; f–g, h, i = four positional isomers of chloro quinoxalinobenzothiazine; j, k, l = bis(3-chlorophenyl)triazene; m = unknown; n = bis(3-chlorophenylbutyl)triazene.

variance in the direction of the development increases, as predicted by eqn. 9. The diffusion coefficient and the contribution to the plate height can be calculated from these data; the results agree well with theoretical predictions.

Fenimore published a chromatogram (ref. 23, Fig. 9) showing a spot capacity of *ca.* 7 for a 3-cm long development instead of a theoretical capacity which is difficult to calculate exactly because of the lack of data but is about 17 (Table IV) for $\sigma_i = 0$ and is reduced to only 9 with a sample spot size corresponding to $\sigma_i = 0.1$ mm. Prosek and Kucan[26] showed a chromatogram with an approximate spot capacity of 16 instead of a theoretical value of 21. Siouffi *et al.*[24] published other data which show that it is possible to achieve values of the spot capacity of around two thirds of the theoretical value calculated with $\sigma_i = 0$, without using a sophisticated sample system, if the development length exceeds 5 cm.

Finally experiments were made using Merck Si 60 TLC and HPTLC plates developed with carbon tetrachloride–chloroform to analyze chlorophenyl triazenes and chloroquinoxalino benzothiazines. The results are given in Table VIII and Fig. 6 shows one of the chromatograms obtained. This figure also illustrates the fact that in spite of the efforts made to separate the largest possible number of compounds on this plate, it is difficult to spread more than 13 spots on a TLC plate with a spot capacity of 16. The data in Table VIII demonstrate good agreement between spot capacities predicted and measured. The differences may be ascribed to the sources of band broadening originating in the spectrophotodensitometer (spot size and response time)[25].

CONCLUSION

TLC offers an excellent level of performance for easy separations. For peak capacities around 20 or below it can be considered as competitive with column chromatography in terms of spot capacity per unit time (*cf.*, Table IX). The analysis time increases very rapidly, however, for spot capacities beyond *ca.* 25; it increases much faster than the necessary plate number, while the characteristics of the optimum plate become rapidly unacceptable, which is in striking contrast to what happens in column

TABLE VIII

COMPARISON BETWEEN PREDICTED AND MEASURED SPOT CAPACITIES

Development length (cm)	*Particle size (μm)**	*Solvent composition: carbon tetrachloride–chloroform*	*Spot measured***	*Capacity predicted****
6	11	80:20	18	19
6.5	11	80:20	16	19
5.5	5	60:40	16	20
4.5	5	70:30	17	19
5	5	70:30	17	20

* Data from Merck.

** From the width of well resolved peaks.

*** Using the same values of parameters as for Table IV.

TABLE IX

COMPARISON OF ANALYSIS TIME IN TLC AND COLUMN LIQUID CHROMATOGRAPHY (CLC)

Spot or peak capacity required	*Conditions in TLC**			*Conditions in CLC***				*Necessary plate number*
	L (cm)	*d_p (μm)*	*t_A (min)*	*L (cm)*	*d_p (μm)*	*ΔP (atm)*	*t_A (min)*	
10	0.5	5	0.14					324
15	1.6	5	0.71	1.2	5	4.4	1.00	784
20	5	7	5.0	2.2	5	8	1.8	1444
30	30	20	62	4.9	5	18	4.0	3364
50	Practically impossible			13	5	49	11.1	9604

* Analysis time = development time. $A = 1$; $C = 0.01$; $\gamma = 0.7$; $\theta = 120$ cm/sec; $D_m = 5 \cdot 10^{-6}$ cm²/sec; $z_0 = 0.2$ cm.

** Analysis time = elution time for $k' = 6.4$. $A = 1$; $C = 0.01$; $\gamma = 0.7$; $\eta = 0.6$ cP; $k_0 = 1 \cdot 10^{-3}$; $D_m = 5 \cdot 10^{-6}$ cm²/sec. As these analyses are easy they are all carried out at $v = 15$ and $h = 2.71$, not at the optimal flow velocity ($v_0 = 2.8$; $h_m = 1.94$).

chromatography[20]. Unfortunately, only very few compounds can be separated with a spot capacity of 20, because it is difficult to spread the components of a mixture evenly. Much attention should thus be given to system selectivity, which explains the traditional use of complex solvent mixtures in TLC.

There is, however, a unique advantage of TLC over column chromatography which is not often used, namely the possibility of carrying out two successive developments in two perpendicular directions, using two different retention mechanisms. The performance achieved would then be really high, because with a development time twice as long as in one-dimensional TLC a spot capacity of the order of the square of that achieved in TLC could be expected. This rapidly becomes very large. This problem will be discussed in a forthcoming paper[27].

It should be noted that the spot capacity is formally identical with the separation number[28], although the method of calculation is different. The results obtained here confirm the range of experimental values obtained by Kaiser[28], *i.e.*, 9–15, but not his estimates of the theoretical potential of HPTLC (*i.e.*, up to 40), which stemmed from excessive enthusiasm.

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