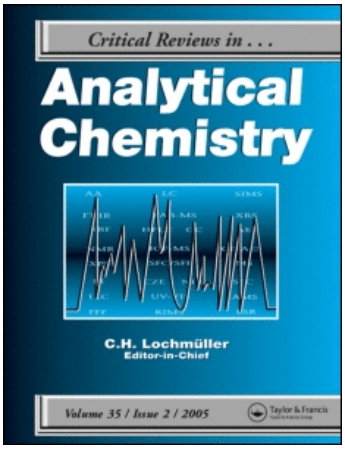

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

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To cite this Article Jupille, Thomas H. and Perry, John A.(1977) 'High-Performance Thin-Layer Chromatography: A Review of Principles, Practice, and Potential', Critical Reviews in Analytical Chemistry, 6: 4, 325 — 359
To link to this Article: DOI: 10.1080/10408347708542695
URL: http://dx.doi.org/10.1080/10408347708542695

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY: A REVIEW OF PRINCIPLES, PRACTICE, AND POTENTIAL

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INTRODUCTION

Chromatography is the separation of a mixture by differential migration resulting from the different distributions of its components between immiscible stationary and mobile phases. The most fundamental classification of chromatographic methods[1] depends on whether the mobile phase is in a gas or a liquid state. Further distinctions can be made on the basis of the state of the stationary phase and the mechanism of distribution. For the purposes of this discussion, however, the most convenient classification is based on the experimental technique used (Figure 1).

A. Brief Classification of Chromatographic Methods

Gas chromatography (GC) – A sample (as a vapor) is carried by a moving gas through a column containing stationary material. The stationary material selectively retards the passage of different sample components. The retardation may be the result of adsorption on a solid surface (gas-solid chromatography, GSC) or partition between the moving gas and a stationary liquid coated onto the column walls or onto an inert solid support (gas-liquid chromatography, GLC).

Liquid chromatography (LC) – A sample (in solution) is carried by a moving liquid solvent through a column packed with stationary material. The stationary material may retard the passage of the sample by adsorption onto a solid surface (liquid-solid adsorption chromatography, LSAC), by ionic attraction to charged groups bonded onto a solid surface (ion-exchange chromatography), or by selective exclusion from pores in a solid matrix (exclusion chromatography, gel filtration, or gel permeation). Alternatively, the sample components may be retarded by partition between the moving solvent and an immiscible stationary liquid coated on or bonded to a solid support (liquid-liquid chromatography, LLC). "Normal" partition assumes the stationary phase to be more polar than the mobile phase; "reverse phase" partition assumes the mobile phase to be more polar than the stationary phase.

Thin-layer Chromatography (TLC) – A sample (in solution) is carried by a flowing liquid through a thin bed of stationary material deposited on a rigid backing. The choice of a stationary phase and a separation mechanism is similar to that in LC.

In the past year, the term "high performance" has been applied to several TLC techniques;[2-4] however, the term has only been loosely defined. We will consider parallels among thin-layer, gas, and liquid chromatography as a base for measuring and judging performance in TLC. We will then consider various "high-performance" TLC techniques in view of the criteria used in other forms of chromatography.

B. TLC in Relation to Other Chromatographic Methods

Chromatographic techniques are complementary; it seems unlikely that any single technique would prove suitable for all separation

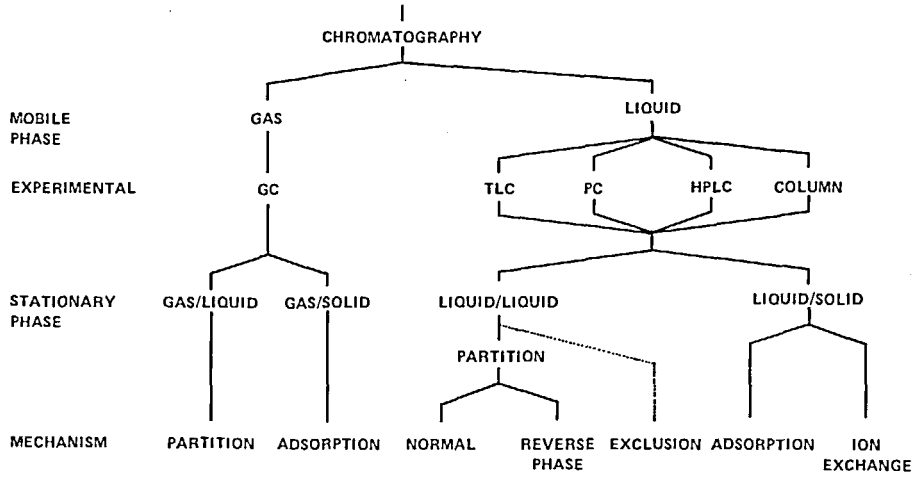

FIGURE 1. Chromatographic techniques can be classified in a variety of ways. In this review, we concentrate on differences in experimental technique.

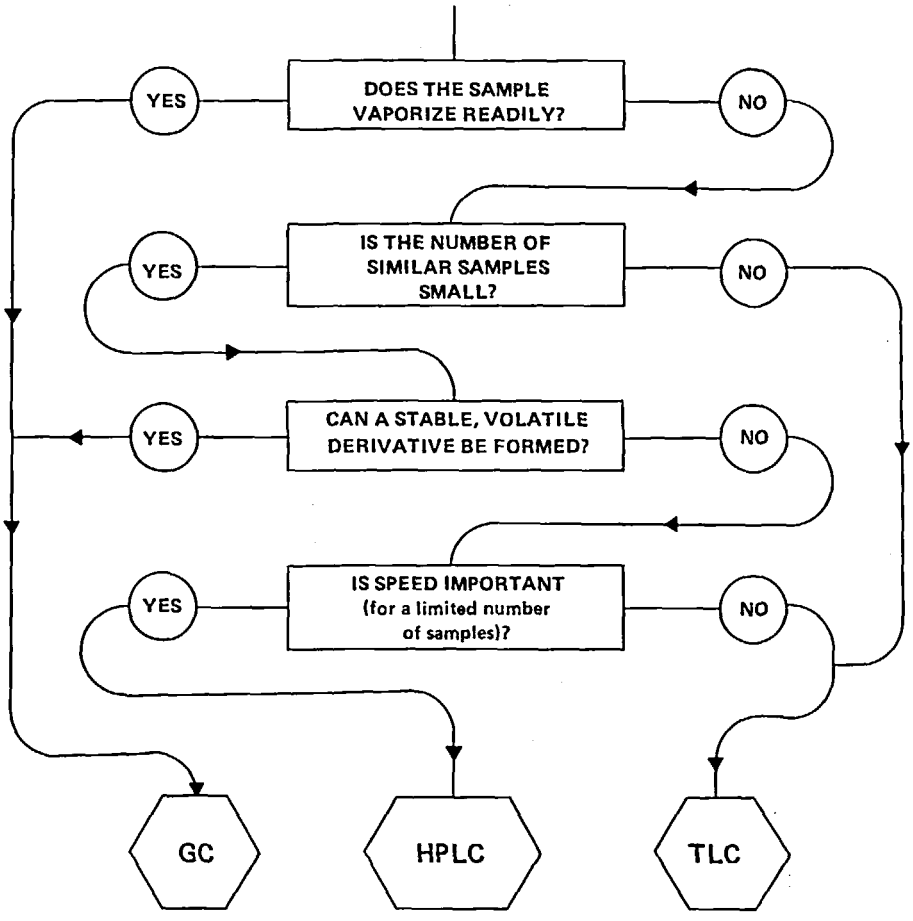

FIGURE 2. Chromatographic techniques are complementary. GC is capable of fast, efficient separation, but is restricted to use with volatilizable compounds. HPLC provides rapid single-sample analysis, while TLC is capable of processing a number of samples simultaneously.

problems. The current conventional wisdom on the relative merits of GC, LC, and TLC is summarized in Figure 2. This diagram reflects a conviction that GC still provides the best available resolution and sensitivity.

GC has been (and remains) the chromatographic method of choice, largely because of a 10-year lead in instrument development.[5] However, its use has been diminishing and will continue to diminish with the continuing advances in LC instrumentation and technology. The advantages of GC are offset by a major disadvantage: Samples for GC must possess some measurable degree of volatility at conveniently attainable temperatures (e.g., up to about 350°C). Unfortunately, most organic compounds, do not meet this criterion. Such compounds can be gas chromatographed only after conversion to chemical derivatives which are more volatile and/or more thermally stable than the parent compounds.

LC does not share the volatility/thermal stability requirement of GC; separation by LC merely requires that sample components be soluble in an appropriate solvent. In other matters, however, GC and LC share many features.[6] For example, the use of columns provides the ability to control mobile-phase velocity over a wide range by varying the inlet pressure. It also allows convenient use of an appropriate detector at the column outlet for quantitation of sample components as they elute from the chromatographic system.

Column methods also have drawbacks when compared to open-bed techniques. For example, the use of a column limits the chromatographic system to one-at-a-time (sequential) sample pro-

cessing. Thus, the total analysis time for n number of samples is at least n times the single-sample analysis time. In addition, detection in column separations is limited to those components which elute from the column. This can lead to considerable uncertainty in the analysis of unknown samples.

TLC is complementary to column techniques in the following respects.[7] The conventional TLC apparatus, a rectangular plate coated on one side with a thin layer of stationary material, allows simultaneous separation of a number of samples (parallel processing). The result is that multiple analyses can be carried out in a total time comparable to that of single-sample analysis. In addition, because in most cases separated components are visualized directly on the thin-layer plate (rather than being eluted from the bed before detection), all of the sample components can be accounted for. The accessibility of the bed in TLC also offers advantages in detection methods; a variety of reagents may be used and processes carried out for component visualization, identification, and detection without the need for hardware modification or real-time analysis. From the early development of TLC in the late 1950s until the advent of high-performance liquid chromatography (HPLC) in the late 1960s, TLC was the preferred method of analytical chromatography using a liquid mobile phase.[8] Recently, however, the tendency has been to consider TLC as a "second-class" technique – inexpensive and easy to use, but not capable of accomplishing difficult separations.[9] However, the perceived limitations of TLC as compared to HPLC are not inherent to the TLC method. They merely reflect the fact that TLC, as it is conventionally carried out, is less efficient than HPLC. This need not be true; with the advent of improved TLC plates and the recognition and use of techniques which magnify the efficiency of TLC systems, the performance of TLC has become comparable to that of GC and HPLC.

C. The Evolution of Chromatography

The history of chromatography[10] is usually traced to Michael Tswett's separation of plant pigments during the early 1900s, although work which can be called chromatography was done as early as 1855. Column chromatography was used sporadically during the first third of this century, but the modern development of chromatography and chromatographic theory began with the description of partition chromatography by Martin and Synge in the early 1940s.

Martin and Synge's plate model explained chromatography (a continuous process) in terms of a series of discrete equilibrations between the stationary and mobile phases and the sample. The plate model allowed correlation of sample migration velocity with an easily understandable parameter, the partition coefficient of the sample between the two liquid phases. In addition, it provided an accurate model for the shape of the zone profile during development (Gaussian) and a relationship between the degree of zone broadening during development and the migration distance (or time), the theoretical plate number (N) defined by analogy with distillation theory as the number of equilibrations which would give rise to the observed sample zone profile. Despite these advantages, the plate model is unsatisfactory in several respects. The most serious shortcoming of the plate model is that it fails to provide a correlation between the theoretical plate number and the working parameters of the chromatographic system (mobile phase velocity, bed length, stationary particle size, etc.).

As a result of their work in partition chromatography, Martin and Synge developed the technique of paper chromatography (PC), in which a sample (in solution) is carried through and along a sheet of paper (usually filter paper) by a liquid solvent flowing (usually) by capillarity.[11] The sample components are retarded as the result of partition between the moving solvent and stationary water bound to the cellulose fibers. PC combined ease and economy of operation with a degree of separating ability hitherto unavailable in chemistry and biochemistry. By the early 1950s PC had grown into an indispensible, ubiquitous technique in these disciplines. Despite its great utility, however, PC suffers from a number of disadvantages. It is slow and inefficient (a fact which led to its virtual replacement by TLC during the 1960s). Furthermore, PC does not allow convenient variation of mobile-phase velocity (which depends on the viscosity of the solvent and the permeability of the paper) or of the stationary-phase parameters (size and shape distribution of the fibers, water content, etc.). On the other hand, it does allow relatively easy modification of the mobile-phase composition. As a result, most of the attention devoted to improving PC

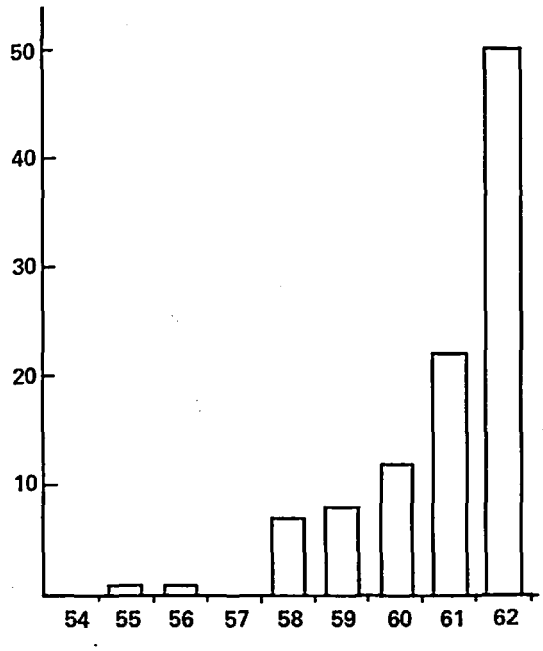

FIGURE 3. The number of gas chromatography-related patents issued annually from 1954 through 1962 illustrates the rapid development of GC instrumentation during this period. This growth spurred the development and application of chromatographic theory.

centered on selectivity ("How far apart can we get the zones?") rather than efficiency ("How narrow can we keep the zones as they separate?"). Theoretical and practical advances in chromatographic efficiency awaited the impetus of the explosive growth of GC.

GLC was suggested by Martin and Synge in the early 1940s and finally developed into a viable technique by James and Martin in 1952.[12] The first commercial gas chromatograph (the Perkin Elmer® 154) was introduced in 1955, and the development of GC instruments and theory was rapid during the next decade (Figure 3).[18] The early development of GC, like the development of PC, emphasized selectivity. (Over 400 materials have been used as GC stationary phases.[13] Of these, 225 were used generally enough to be included in McReynolds'[14] study of stationary-phase polarity. It has been suggested that only two dozen are really necessary for most GC work.)[15] However, the flexibility of GC in terms of flow rate, particle size and shape, and column length allowed relatively easy modification of GC conditions in the quest for greater efficiency.

The accumulating data allowed the formulation of an expression relating chromatographic efficiency to the characteristics of the column.[16] The Van Deemter Equation formed the basis for theories of band spreading in chromatography which have been successfully applied to all types of chromatographic processes.[17] During the 1950s, however, band spreading theories were developed and applied to GC almost to the exclusion of the development of other techniques. The rapid development and application of GC theory made GC the method of choice wherever it was even remotely applicable. Much effort was (and continues to be) devoted to the formation of gas-chromatographable chemical derivatives of nonvolatile or unstable organic compounds.[19]

TLC on loose layers was described by Ismailov and Schreiber in the 1930s.[20] However, the direct ancestor of TLC as it is practiced today is the "chromatostrip" developed by Kirchner and Miller in 1952. The reproducibility and efficiency of early TLC plates or strips was poor, however, and the technique remained obscure until the late 1950s, when it was standardized by Stahl and applied to a variety of separation problems. The use of TLC grew rapidly after 1960, much of its growth occurring at the expense of PC. In most cases, TLC was viewed (naturally enough, considering the experimental similarities) as merely a faster, somewhat more efficient version of PC.[22] Liquid-column chromatography dates back to the work of Michael Tswett. In fact, until the late 1960s most LC separations were carried out in columns which differed little from Tswett's. Major changes in LC arose in the early 1960s with the realization that the principles of GC column technology were applicable to the design of LC columns.[23-25] The use of LC gathered momentum in the late 1960s with the development and widespread acceptance of new techniques which were termed HPLC:

> Of the various forms of chromatography, that with a liquid eluant came first. Its use became established during the 1940's, and it has been used extensively for routine purposes with comparatively little further development. Gas chromatography grew out of liquid chromatography in 1952 and in the last fifteen years, its technique and its principles have been developed to a far greater extent than those of liquid chromatography. There are recent signs, however, that liquid chromatography is beginning to develop again, making use of the principles and practices which have been acquired in connection with gas chromatography.[26]

We can paraphrase this statement to say that there are recent signs that TLC is beginning to develop again, making use of the principles and practices which have been acquired in connection with HPLC.

D. Factors Influencing Chromatographic Performance

Resolution of sample components is the primary object of chromatography. If we consider two chromatographic zones, each of width w and separated by a distance ΔX, we can define resolution (R_s) as

$$R_s = \Delta X/w \quad (1)$$

(see Figure 4.) The expression of resolution will vary with assumptions made about the measurement of width and separation, and several criteria for "complete" resolution have been proposed.[27,28] In qualitative terms, however, all such interpretations condense to the statement that two zones are resolved if they are farther apart than they are wide. Resolution can be improved by increasing the separations between zones (selectivity) and/or by decreasing the zone width (efficiency). As a crude description, we can say that selectivity in chromatography (because it deals with differences in distribution coefficients between sample components) is a thermodynamic variable. On the other hand, efficiency (because it deals with the dynamic process of band spreading during separation) is a kinetic variable.[29]

The selectivity of a chromatographic system is strongly dependent on the nature of the sample components to be separated. As a result, statements made about selectivity toward a given set of components will not generally be applicable to other sets of components. However, efficiency is primarily determined by the physical characteristics of the chromatographic system used. It is generally only slightly dependent on the identity of the sample. Without denying the importance of selectivity to chromatographic resolution, we will focus our attention on the efficiency of chromatographic systems as a measure of their performance. Indeed, we use the terms "high performance" and "high efficiency" interchangeably.

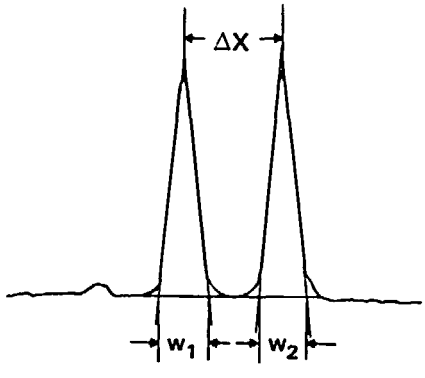

FIGURE 4. Resolution in chromatography is the ratio of separation to zone width.

As sample zones migrate through a chromatographic system, they broaden. The profile of a sample zone is ideally described as a Gaussian (bell-shaped) curve.[30] As such, it is characterized by a maximum height (in chromatographic terms, concentration of sample molecules at the zone center) and a standard deviation (σ). The standard deviation is measured as the distance from the apex of the distribution to either inflection point. The degree of zone spreading per unit migration distance (or migration time), X, is expressed by the theoretical plate number, N, defined as

$$N = (X/\sigma)^2 \quad (2)$$

N no longer has any connotation of number of discrete equilibrations as it had in plate theory.[31]

The efficiency of a system is also often expressed as the plate height, H, (also called the height equivalent to a theoretical plate, HETP), defined as the ratio of the zone variance, σ^2, to the migration distance, X:

$$H = \sigma^2/X \quad (3)$$

$$H = X/N \quad (4)$$

Grushka et al.[32] have recently presented a qualitative description and discussion of band spreading theories in chromatography. The model presented here is based primarily on the random-walk treatment of Giddings.[29] It serves to identify and describe the source of band spreading in chromatography and can be applied to the (approximate) prediction of the optimum performance attainable in TLC. This value can be compared with currently accepted performance standards in GC, LC, and TLC.

The use of plate height, H, as a measure of

efficiency is advantageous in the development of chromatographic zone-spreading models despite protests[33] that it unnecessarily complicates chromatographic calculations. If a number of independent effects are assumed to contribute to zone broadening, then, according to the laws of statistics, the final zone variance is the sum of the contributing variances:

$$\sigma^2 = \sum_i \sigma_i^2 \quad (5)$$

Because H is a function of zone variance, the total plate height in a chromatographic system is the sum of the plate height contributions from the various independent zone-broadening processes:

$$H = \sum_i H_i \quad (6)$$

The classical theory of Van Deemter et al.[16] considered the effects on gas-chromatographic peak widths of three independent spreading mechanisms. **Eddy diffusion** – Eddy diffusion is the result of unequal flow velocities/path lengths experienced by different sample molecules in a packed bed. The eddy diffusion term (A) in the classical Van Deemter equation is independent of the mobile phase velocity. The significance of eddy diffusion as a contribution to plate height in TLC is discussed in Section II.C. **Molecular diffusion** – Molecular diffusion is the result of diffusion of sample molecules during their residence in (usually) the mobile phase (molecular diffusion in the stationary phase is usually not the principal mechanism of spreading in GC. This statement probably also holds true in liquid-solid chromatography; however, it may be a significant factor in some forms of liquid-liquid chromatography). The molecular diffusion term (B/v) of the classical Van Deemter equation is considered to be inversely proportional to the mobile-phase velocity (v). This expresses the fact that, at low mobile-phase velocity, a molecule spends a considerable residence time in the mobile phase during the course of a given migration; thus, it has ample opportunity to diffuse. **Resistance to mass transfer** – Resistance to mass transfer is the result of the finite time required for sorption and desorption of sample molecules. This prevents the attainment of equilibrium in a moving system. Thus, the mass transfer term of the Van Deemter equation (Cv) is directly proportional to the mobile-phase velocity (the higher the velocity, the less the opportunity for equilibration).

The classical Van Deemter equation expressed plate height as the sum of independent contributions,

$$H = A + B/v + Cv \quad (7)$$

This is a form which is an adequate description of most gas-chromatographic systems. However, when very high mobile-phase velocities are used in GC, and when the Van Deemter equation is applied to liquid-chromatographic systems, it becomes apparent that in many cases the magnitude of the A term is smaller than expected.[29,34,35] As was pointed out by Giddings,[29] this discrepancy is readily explained by the fact that the A and the C terms are not, after all, independent. In effect, the existence of resistance to mass transfer in the mobile phase limits eddy diffusion and vice versa. The net effect of this "coupling" can be approximately expressed by adding the A and C terms harmonically rather than linearly to give the coupled plate-height equation

$$H = B/v + C_s v + 1/(1/A + 1/C_m v) \quad (8)$$

in which resistance to mass transfer is divided into a stationary-phase contribution ($C_s v$), which is independent of eddy diffusion and a mobile-phase contribution ($C_m v$), which is coupled with eddy diffusion (Figure 5).

The A, B, C_s, and C_m terms can be evaluated in the following manner for column systems[29] (constant bed length, constant mobile-phase velocity, and variable migration times for different sample components are assumed):

$$A = 2\lambda d_p \quad (9)$$

where d_p is the average stationary-phase particle diameter, and λ is a geometrical factor reflecting packing irregularities.

$$B = 2\gamma_m D_m \quad (10)$$

where γ_m is an obstruction factor describing the limitation on free diffusion in a packed bed, and D_m is the diffusion coefficient of the sample in the mobile phase.

$$C_s = 2R(1 - R)d_f^2/D_s \quad (11)$$

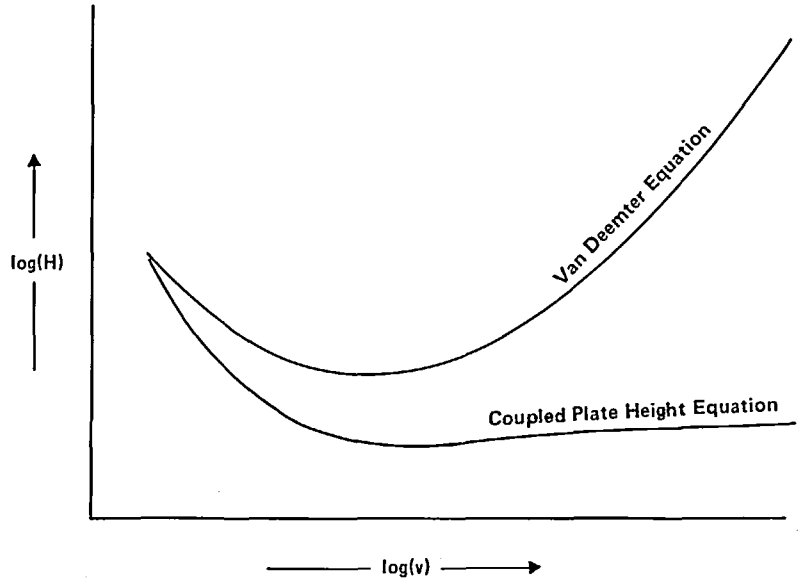

FIGURE 5. The classical Van Deemter equation, H = A + B/v + Cv, predicts a linear increase in plate height as the mobile-phase velocity is increased past its optimum value. However, the behavior of liquid mobile-phase systems is better described by the coupled plate-height equation, H = B/v + 1/(1/A + 1/Cv), which takes into account the interdependence of eddy diffusion and resistance to mass transfer in the mobile phase.

where R is the relative migration velocity (see below) of the sample, d_f is the film thickness of the stationary phase, and D_s is the diffusion coefficient of the sample in the stationary phase.

$$C_m = 2W(1 - R)^2 d_p^2/D_m \quad (12)$$

where W is a constant which corrects for packing structure.

E. High Performance in Chromatography

The definition of high performance in chromatography is, of necessity, highly subjective. We will assume here (admittedly arbitrarily) that high performance implies, as a minimum, a fivefold improvement in efficiency over conventional techniques. For example, adequate conventional GC performance is represented by a 6 ft long packed column generating 500 to 800 theoretical plates per foot,[124] some 3000 to 5000 plates overall. Thus, our criterion of fivefold improvement would define high performance in GC as the capability of generating 15,000 to 25,000 theoretical plates. Likewise, the 200 to 500 theoretical plates typical of conventional liquid column chromatography[125] suggest that high-performance LC can be defined in terms of the ability to generate a minimum of 1000 to 3000 theoretical plates. This definition fits well with the data provided in a recent compilation of liquid chromatograms.[116]

Another approach to the expression of efficiency in LC is an emphasis on delivery – the number of theoretical plates generated per unit time. An examination of the LC separations cited above[116] suggests that high performance can be defined in these terms as the ability to deliver at least one plate per second.

Conventional TLC is typically capable of generating 1000 to 2000 theoretical plates (e.g., a 1 cm in diameter spot at a distance of 10 cm from the origin represents 1600 plates). Thus, our criterion of fivefold improvement suggests that HPTLC can be defined by the capability of generating at least 5,000 to 10,000 theoretical plates. Alternatively, using delivery as a criterion and high-performance LC as a guide, we suggest that HPTLC can also be defined as the ability to deliver at least four plates per second.

II. PERFORMANCE IN THIN-LAYER CHROMATOGRAPHY

A. Basic TLC Definitions

A TLC plate consists of a thin bed of stationary material coated onto a rigid backing. In use, a small volume of a solution (in a suitable volatile solvent) of the sample is applied as a spot on the surface of the thin layer a short distance from the bottom edge of the TLC plate. The position of this spot is the origin of the TLC plate. The bottom edge of the plate (but not the spot) is then immersed in the mobile-phase solvent, which begins to travel through the layer as the result of capillarity (development of the plate). The boundary of the wetted area farthest from the solvent reservoir is the solvent front. Although the backing is usually a rectangular plate, other geometries can be and have been used (see below). Similarly, development need not be carried out by capillarity in a vertical direction. The above description defines what might be called "classical TLC" as developed at Stahl,[21] the most commonly used form of TLC.

Development is not carried out in an open atmosphere because of excessive solvent evaporation from the wetted surface of the plate. In the most common arrangement, development is carried out in a large parallelepiped tank containing a shallow pool of solvent at the bottom. The atmosphere of the tank is preequilibrated with the solvent to ensure saturation with solvent vapors. This type of developing tank is called an N-chamber ("normal"). A common alternative to the N-chamber consists of a cover plate separated from the thin layer by spacers and held in place by clips. The bottom edge of the resulting S-chamber ("sandwich") is immersed in an external solvent reservoir. The small atmosphere volume of the S-chamber limits solvent evaporation from the plate surface[37] (however, a significant amount of evaporation does occur; this is the basis for the improved performance of the S-chamber as compared to the N-chamber in many applications).[37,38]

As the solvent advances through the thin layer, its velocity decreases. To a first approximation, the velocity of solvent advance is inversely proportional to the distance from the reservoir to the solvent front (Poiseuille's Law).*[39,40] For most purposes, the solvent velocity at any given instant is constant over the entire plate.** A necessary consequence of Poiseuille's Law is the proportionality of development time to the square of development distance in TLC. A threefold increase in TLC development distance thus requires a ninefold increase in development time. In conventional TLC (as in PC), the solvent velocity is not subject to direct experimental control. It is determined by the solvent viscosity and the mean particle diameter (and particle size range) of the bed.

Sample components are retarded by interaction with the stationary phase. As a consequence, they migrate at some fraction of the mobile-phase velocity. To a first approximation, this relative migration velocity (R) is equal to the observed relative migration distance (R_f) (Figure 6).*** In practice, R_f is determined by the ratio of the spot migration distance to the distance from the origin to the solvent front. The relative migration velocity depends on the distribution of sample molecules between the mobile and stationary phases. For example, a component contained entirely in the mobile phase would be carried forward at exactly the mobile-phase velocity; it would have a relative migration velocity of unity. Similarly, a molecule contained entirely within the stationary phase would not move at all; it would thus have a relative migration velocity of zero. A component equally divided between the phases would have a relative migration velocity of one half.

Relative migration velocity can be related to another sample parameter, the distribution factor or capacity factor (k'). The distribution factor is defined as the ratio of the quantity of sample in the stationary phase to the quantity of sample in the mobile phase. It is related to relative migration velocity by

*This approximation neglects the retarding effect of the pull of gravity in conventional ascending chromatography. However, in most cases, the effect of gravitation is negligible as compared to the magnitude of the capillary forces in TLC. This is in contrast to PC, in which the capillary forces are considerably weaker and for which numerous descending chromatography schemes have been devised.

**This is strictly true only if the concentration of solvent is constant over the entire plate. In practice, the solvent concentration is found to decrease somewhat near the origin, indicating that the bulk solvent velocity is somewhat slower than the solvent front velocity.[41-43]

***Because solvent concentration decreases near the front, R_f is typically approximately 15% smaller than R.[29]

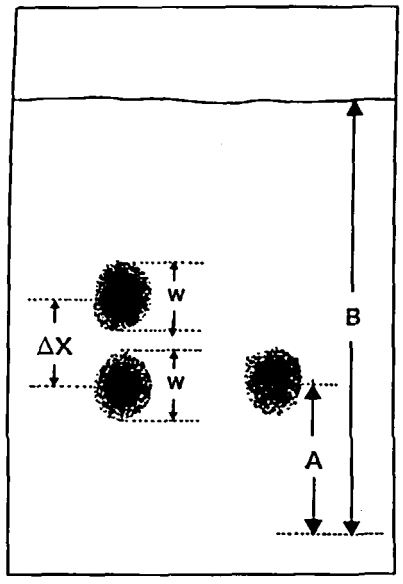

FIGURE 6. Resolution in TLC (R_s) is the ratio of separation to spot width. Relative migration distance (R_f) is the ratio of spot migration distance to solvent migration distance.

$$k' = (1 - R)/R \quad (13)$$

The distribution factor is also related to the thermodynamic distribution coefficient (k) by the phase ratio, ϕ, (ratio of volume of stationary phase to volume of mobile phase)

$$k' = k\phi \quad (14)$$

B. Resolution, Selectivity, and Efficiency in TLC

As sample components migrate during development, they separate from one another and they broaden, ideally assuming a Gaussian profile. Resolution in TLC (as in other forms of chromatography) is defined as

$$R_s = \Delta X/w \quad (1)$$

$$R_s = \Delta X/4\sigma \quad (1a)$$

A number of criteria have been proposed for "complete" resolution in chromatography.[27,28] The most straightforward (and most commonly used in TLC) is that resolution is complete when R_s is greater than or equal to unity. Other possibilities include the suggestion that resolution is complete if the separation is equal to or greater than that at which the $1/\sqrt{e}$ times maximum points on the adjacent bands overlap (this by analogy with the Reyleigh criterion for resolution in optics;[44] for peaks of equal width, this implies a separation greater than or equal to 3 σ, (as opposed to greater than or equal to 4 σ for the conventional definition). It has also been pointed out in connection with preparative work that if two "completely" resolved peaks (R_s = 1) are separated at exactly the minimum of the trough, each "pure" component would be contaminated with 2.3% of the other (assuming equal peak sizes). Thus, the criterion that R_s must be equal to or greater than 1.5 is often used to judge complete resolution. In this case, the cross-contamination of equal sized peaks would be limited to 0.14%. Charts of resolution required for given purity levels in preparative chromatography are available.[45] Regardless of the criterion used for completeness, the fact that resolution is a ratio of separation to spot width is often pointed out in the TLC literature[46] and is also often ignored.[22,47,48] Therefore, it is reemphasized that resolution can be improved in the following two ways: by increasing separation and by decreasing spot width.

The center-to-center separation between spots on a TLC plate depends on the length of the bed traversed by the front, the average relative migration velocity of the compounds of interest, and the selectivity of the system toward the compounds of interest. Selectivity in TLC is conveniently expressed as the migration ratio (r) of the components of interest

$$r = R_1/R_2 \quad (15)$$

where the first and second spots are identified so that r is greater than or equal to unity.

The migration distance of a spot (X) is proportional to its relative migration distance and to the solvent front migration distance (X_s).

$$X = R_f X_s \cong R X_s \quad (16)$$

The separation between two spots A and B (ΔX_{AB}) is the difference in migration distances.

$$\Delta X_{AB} = X_s R_B (r - 1) \quad (17)$$

The spot width also depends on the length of the bed traversed by the solvent front and the relative migration velocities of the components of interest, as well as the efficiency of the system as measured by the plate height.

$$w = 4\sqrt{HRX_s} \quad (18)$$

If we assume equal widths for adjacent spots, resolution can be expressed as

$$R_s = \tfrac{1}{4}(r - 1)\sqrt{RX_s}/\sqrt{H} \quad (19)$$

where we have assumed that R_B may be replaced with the average relative migration velocity (R). The last factor ($\sqrt{RX_s/H}$) expresses the efficiency of the TLC system for the compounds of interest – the theoretical plate number for the separation.

C. Factors Influencing TLC Performance

Either the Van Deemter equation or the coupled plate-height equation can be used to describe spot broadening in TLC with suitable modification for TLC conditions. Two types of modification must be made. Unlike sample zones in column chromatography (which must all spend the same total time in the mobile phase during traversal of the column), spots in TLC have a mobile phase residence time which is proportional to their relative migration velocity. Thus, the mobile-phase terms (B term and C_m term) must be corrected by a factor of R to reflect this shortened residence time. Because mobile-phase velocity is not constant, but depends inversely on the distance from the reservoir to the solvent front, the observed plate height in TLC must represent an average plate height for the separation process, $\bar{H}$. This value can be evaluated from

$$\bar{H} = \int_{x_o}^{x_s} H dx \Big/ \int_{x_o}^{x_s} dx \quad (20)$$

Different final results are obtained depending on whether one starts with the classical Van Deemter equation or with the coupled plate-height equation.

If we start with the classical Van Deemter equation, each of the terms may be corrected and evaluated individually. The resulting equation, as given by Stewart for PC,[49] has the form

$$\bar{H} = A + BRX_s/K + (CK/2X_s)\ln(RX_s/X_o) \quad (21)$$

On the other hand, the coupled plate-height equation gives rise to the more complex form

$$\bar{H} = BRX_s/K + (CK/2X_s)\ln[(CK + 2X_sA)/(CK + 2X_oA)] \quad (22)$$

We will use the coupled plate-height equation as a general guide to the relationship between plate height and the parameters describing the TLC system.

Migration distance (X_s) – The first term in the coupled plate-height equation expresses a linear dependence on solvent migration distance. The second term expresses an essentially inverse dependence (the logarithmic factor only changes slowly with migration distance over the typical range of the latter). This implies that there exists an optimum solvent migration distance from the aspect of plate height. The exact value of the optimum migration distance depends on the values assigned to the other system parameters. Figure 7 shows the type of dependence associated with reasonable values of A, B, C, and K.

Relative migration velocity (R) – The dependence of plate height on relative migration velocity is complicated by the dependence of the C term on R. However, the C term can be expressed as the product of R-dependent and R-independent (C′) factors

$$C = C'(1 - R)^2 \quad (23)$$

This allows reexpression of the coupled plate-height equation explicitly for dependence on R

$$\bar{H} = (BX_s/K)R + [(1 - R)^2C'K/2X_s)\ln((C'K(1 - R)^2 + 2X_sA)/C'K(1 - R)^2 + 2X_oA)] \quad (24)$$

A plot of the dependence of $\bar{H}$ on R (Figure 8) shows a very shallow curve. This indicates that for a given system the observed plate height is essentially constant for all sample components. This agrees well with experimental evidence[15] without requiring the conclusion that TLC plate height is determined primarily by eddy diffusion.[54]

Average particle diameter (d_p) – The pseudo-diffusion coefficient describing solvent velocity (K) depends, to a first approximation, on the square of the average particle diameter.[29] Both the A term and the C term also depend on d_p. K, A, and C can each be expressed as the product of d_p-dependent and d_p-independent (K″, A″, and C″) terms

$$K = K''d_p^2 \quad (25)$$

$$A = A''d_p \quad (26)$$

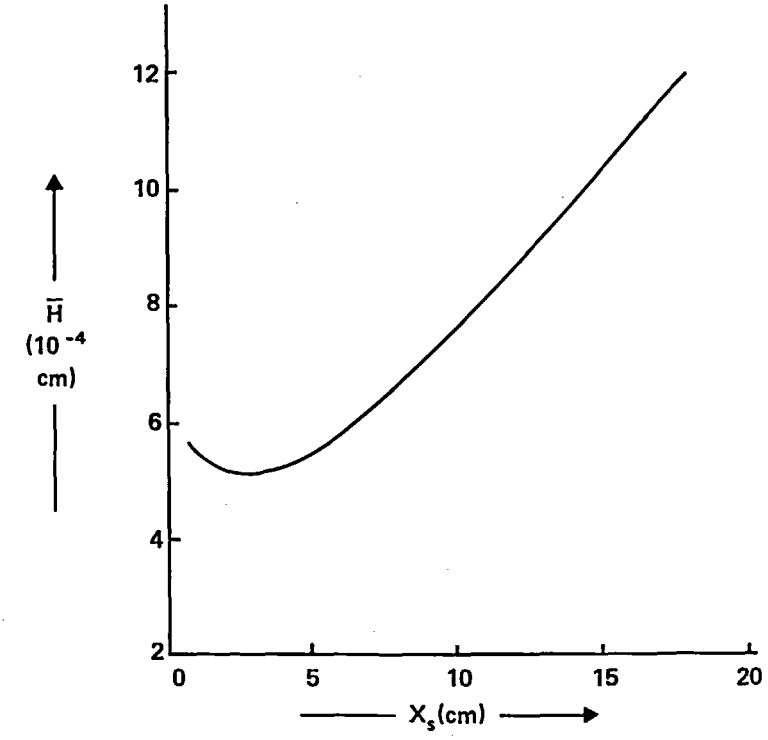

FIGURE 7. The observed plate height in TLC, $\bar{H}$, depends on the solvent migration distance, X_s. The following values are assumed: A = 0.001 cm; B = 1.2 X 10^{-6} cm^2/sec; C = 0.12 sec; K = 0.01 cm^2/sec; X_o = 0.01 cm.

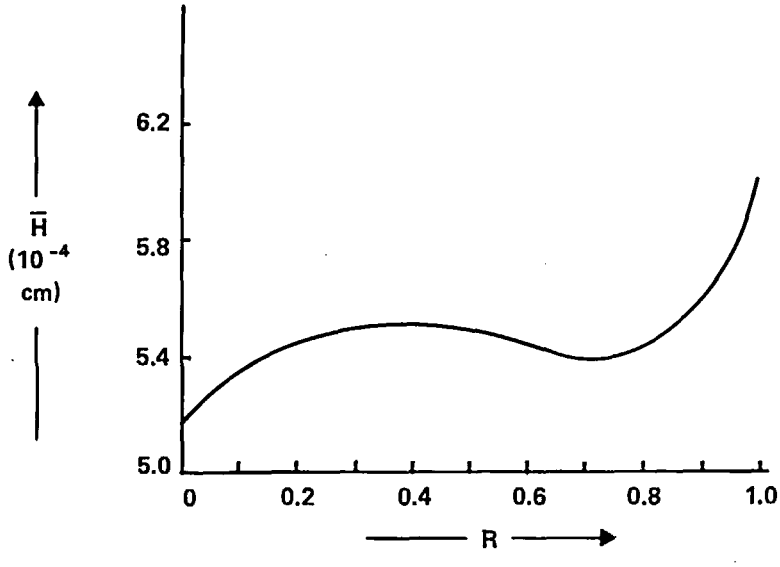

FIGURE 8. The observed plate height in TLC, $\bar{H}$, depends only slightly on the relative migration velocity of the sample, R. The following values are assumed: A = 0.001 cm; B = 1.2 X 10^{-6} cm^2/sec; C = 0.12 sec; K = 0.01 cm^2/sec; X_o = 0.1 cm; X_s = 5 cm.

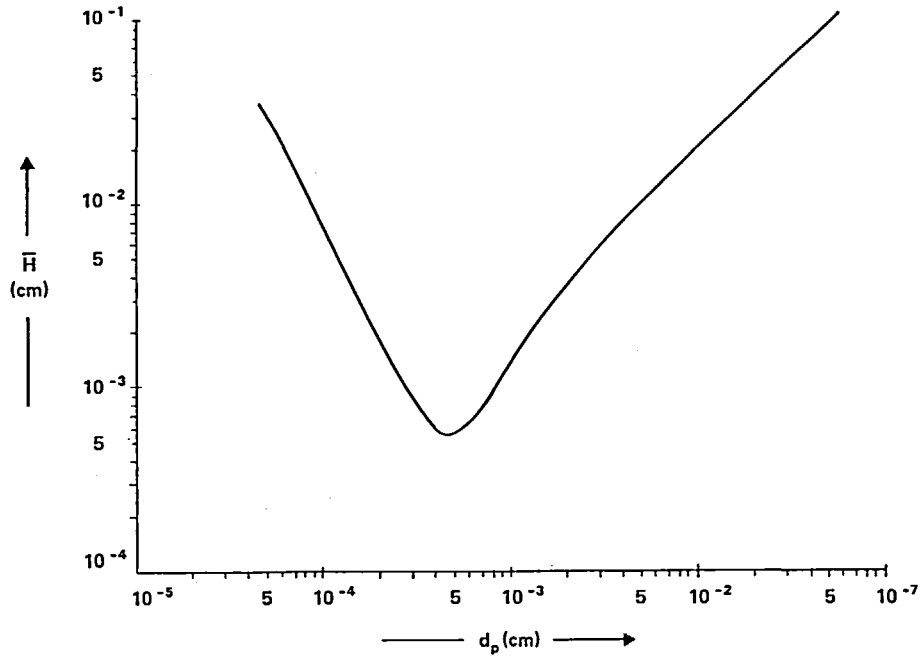

FIGURE 9. The observed plate height in TLC, $\bar{H}$, depends on the average particle diameter of the bed, d_p. The following values are assumed: $A' = 2$; $B = 1.2 \times 10^{-6}$ cm²/sec; $C' = 4.8 \times 10^5$ sec/cm²; $K'' = 4 \times 10^4$ sec^{-1}; $X_o = 0.1$ cm; $X_s = 5$ cm.

$$C = C''d_p^2 \tag{27}$$

This allows us to rewrite the coupled plate-height equation in terms of an explicit dependence on d_p.

$$\bar{H} = (BRX_s/K'')(1/d_p^2) + d_p^4(C''K''/2X_s)\ln[(C''K''d_p^4 + 2X_sA''d_p)/(C''K''d_p^4 + 2X_oA''d_p)] \tag{28}$$

For practical purposes, this may be considered as the sum of a term with a direct dependence on d_p^4 and a term with an inverse dependence on d_p^2. This implies the existence of an optimum particle diameter for any given system. An example of the dependence of $\bar{H}$ on d_p is shown in Figure 9.

D. The Limits of Conventional TLC Performance

The value assigned to the theoretically attainable limit of efficiency in TLC depends on the values assigned to the terms in the reduced plate-height equation. We can obtain a rough estimate of the order of performance expected in TLC on the basis of the following reasonable assumptions concerning these parameters:[29]

1. A term: $A = 2\lambda d_p$. The geometrical factor (λ) has been estimated as (typically) unity; however, smaller values are considered possible for well-made beds. The optimum particle diameter (d_p) has been estimated by numerous authors as being in the 1-μm range; however, a 5 μm-diameter is perhaps more realistic in view of the aggregation problems encountered with very small particles. We can then assign

$$A \cong (2)\,(1)\,(0.0005)\text{cm} = 1 \times 10^{-3}\text{cm} \tag{29}$$

2. B term: $B = 2\gamma_m D_m$. The obstructive factor (γ_m) is conventionally given as being in the range of 0.5 to 1.0, with most values clustering around 0.6. The mobile-phase diffusion coefficient (D_m) depends on the viscosity of the solvent as well as on the molecular weight of the sample. A reasonable estimate for D_m of low molecular weight compounds in organic solvents is on the order of 1×10^{-6} cm²/sec.[50] We can then assign

$$B \cong (2)(0.6)(1 \times 10^{-6})\text{cm}^2/\text{sec} = 1.2 \times 10^{-6}\text{ cm}^2/\text{sec} \tag{30}$$

3. C term: $C = 2W(1 - R)^2 d_p^2/D_m$. The packing factor (W) varies with the quality of the layer and with the particle size distribution; estimates have ranged from 0.002 to 5.0.[34] We will assume a value of 1.0. If, for the sake of

convenience, we assume a sample component whose relative migration velocity (R) is 0.5, we can assign

$$C \cong (2)(1)(1 - 0.5)^2(0.0005\ cm)^2/(1 \times 10^{-6}\ cm^2/sec) = 0.12\ sec \quad (31)$$

4. Pseudo-diffusion coefficient: $K = K''d_p^2$. The value of K'' depends on the solvent viscosity and on the particle size distribution of the layer. For example, K value of 0.05 cm^2/sec was obtained by Stewart[51] on a layer of effective particle diameter approximately 0.001 cm using carbon tetrachloride. This suggests $K'' \cong 5 \times 10^4\ sec^{-1}$; we will use this value as typical. We can then assign

$$K \cong (5 \times 10^4\ sec^{-1})(0.0005\ cm)^2 = 0.01\ cm^2/sec \quad (32)$$

These values allow calculation of an expected plate height for $X_o = 0.5$ cm and $X_s = 5.0$ cm of

$$\bar{H} \cong 6 \times 10^{-4}\ cm$$

This value is 20-fold lower than that which Snyder[52] suggested as the minimum attainable TLC plate height. The latter value was associated with the extrapolation to TLC of empirical relationships from column chromatography and with the use of the "uncoupled" form of the plate height equation.[53] It involves the assumption or conclusion that the dominant contribution to TLC plate height is eddy diffusion;[54] this has since been seriously questioned.[35,56] Our estimate of an attainable plate height, along with reported plate heights in the 10-μm (0.001-cm) range, suggests that conventional TLC (carried out under optimum conditions) may be able to meet the criterion of high performance; i.e., the ability to generate 5000 theoretical plates in a reasonable length of time. In this respect, conventional TLC is analogous to packed-column GC, in which routine performance is approximately 3000 to 10,000 theoretical plates.

As will be discussed in the next section, however, a number of TLC techniques are available which reconcentrate spots (counteracting the effects of broadening) to a greater extent than they decrease center-to-center separation between sample components. The effect of these techniques is to increase the degree of resolution attainable for a given selectivity and plate height (additional effects include improved sensitivity and, often, faster separation). We can define for such techniques a quantity called the equivalent plate number (N_e). The equivalent plate number is the number of theoretical plates required to produce a given resolution at a given selectivity by conventional TLC. In practice, the equivalent plate number may be calculated from a modification of Equation 19 relating efficiency selectivity and resolution

$$N_e = 16R_s^2/(r - 1)^2 \quad (33)$$

For example, a resolution of $R_s = 1.0$ between two components with relative migration velocities differing by 2% (R = 1.02) would require the equivalent of 40,000 theoretical plates; therefore, the equivalent plate number for such a separation is 40,000.

The equivalent plate number is defined in terms of resolution between two sample zones. Thus, it differs fundamentally from the plate number (with no qualifying adjectives), which is defined in terms of migration distance and zone width (per Equations 3 and 4); the latter is directly related to resolution only under ideal conventional TLC conditions.

For a given migration distance (X_s) we can also define an equivalent plate height ($\bar{H}_e$)

$$\bar{H}_e = X/N_e \quad (34)$$

Of course, the equivalent plate height for conventional TLC is identical to the observed plate height previously described and defined. In "nonconventional" TLC systems, specifically those systems leading to the formation of velocity or polarity gradients during development, equivalent plate height differs from plate height defined in the broader sense of degree of band spreading per unit migration distance. Equivalent plate height must be defined by the behavior of a pair of spots. In contrast, plate height can refer to single spot behavior.

Both plate height and equivalent plate height must be further distinguished from apparent plate height in nonconventional TLC. The apparent plate height, (H), is defined by the observed zone width (w) and the migration distance (X)

$$(H) = w^2/16X \quad (35)$$

The relationships among plate height, equivalent plate height, and apparent plate height are

TABLE 1

Plate Height Definitions

Symbol	Name	Definition	Comments
H	Plate height	$H = B/v + 1/(1/A + 1/Cv)$ $H = \sigma^2/X$	In conventional TLC (no gradients) all plate height definitions reduce to this
(H)	Apparent plate height	$(H) = w^2/16X$	Expresses the efficiency in a useful form for situations in which zone width is of primary concern (e.g., when sensitivity is being discussed)
H_e	Equivalent plate height	$H_e = X(r - 1)/16R_s^2$	Expresses the efficiency in a useful form for situations in which resolution is of primary

summarized in Table 1. The distinctions among these quantities reflect the fact that many techniques which reduce spot width also reduce separation. Thus, increases in apparent efficiency (i.e., narrower spots) do not always produce increased resolution. We will use equivalent plates (i.e., resolution) as our criterion for judging performance in TLC.

As TLC performance is improved toward plate heights below 0.001 cm, the influence of extra-bed factors on performance becomes more pronounced. The most obvious of these factors are sample loading and initial spot width. A plate height of 0.001 cm in conventional TLC implies a spot width of 2.8 mm at a migration distance of 5 cm. The influence of initial spot width on apparent plate height is shown in Figure 10. Care must be taken in sample application if the full benefit of high-efficiency systems is to be obtained. When large sample solution volumes must be applied to the TLC plate, results can often be improved by predevelopment of the plate for a short distance with a polar solvent after sample application. The sample is carried forward at the solvent front and is thus reconcentrated into an extremely tight band. A similar effect is more conveniently obtained with plates which incorporate a preadsorbent layer.[57] This zone (typically composed of diatomaceous earth) provides negligible sample retention with most solvents; it allows spot preconcentration without predevelopment.*

Our entire discussion of efficiency in chromatography has implicitly assumed that sample distribution isotherms are linear (i.e., sample distribution coefficients are independent of sample concentration). While this condition is approximately fulfilled at low sample concentrations, it becomes increasingly less so as sample size increases. When the sample load is large enough to significantly affect isotherm linearity, the result is a concentration dependence of relative migration velocity. Different parts of a zone (at different concentrations) migrate at different speeds and excessive zone spreading (tailing or bearding) occurs. In fact, the apparent plate height has been suggested as a basis for quantitation in TLC.[58]

It has been observed (particularly in adsorption chromatography) that in many cases sample capacity can be increased by deactivation of the stationary phase.[52] This is conveniently accomplished by exposure to an atmosphere containing the vapor of a strongly adsorbed component (often water).[59] However, this exposure should be maintained during the entire development because evidence indicates that TLC plates equilibrate rapidly with their surroundings.[60] Unless explicitly noted otherwise, our discussion of high-performance TLC will

*Commercially available from Quantum Industries, Fairfield, N.J.

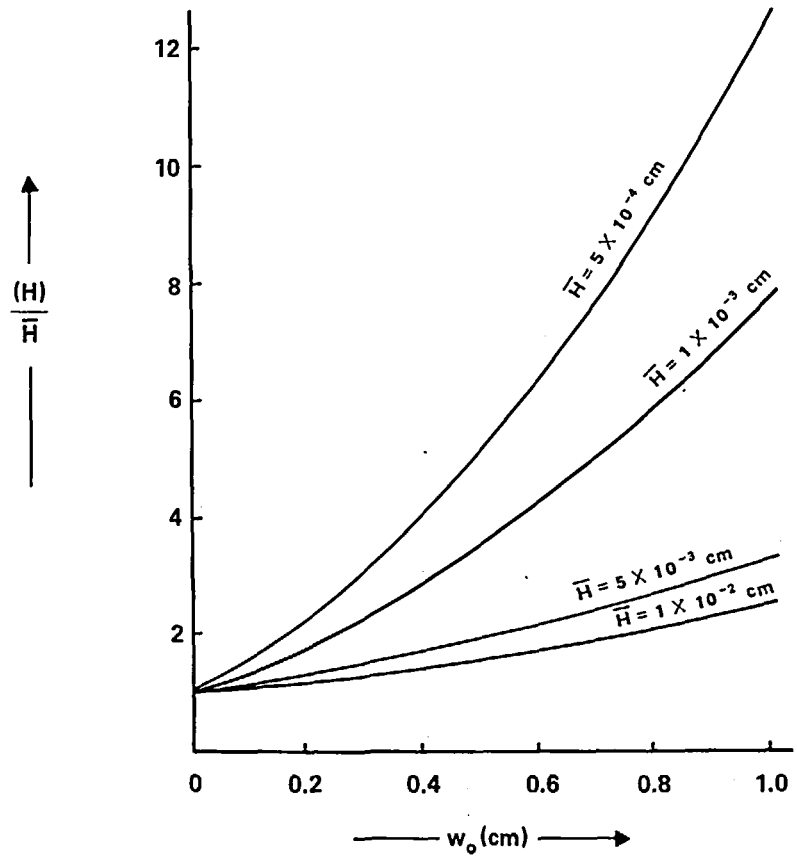

FIGURE 10. Apparent plate height, (H), increases with increasing initial spot width, w_0. The relative deterioration increases as plates become more efficient ($\overline{H}$ decreases).

assume linear distribution isotherms and negligible initial spot widths.

III. THE SEARCH FOR IMPROVED TLC PERFORMANCE

A. Nonconventional TLC Media

The chromatographic format standardized by Stahl remains the basis for the overwhelming majority of TLC separations. Nevertheless, many variations on this format have been made with the object of improving sample handling, loading capacity, or performance.

The most straightforward modification of this kind is cylindrical or tube TLC.[61-64] A "chromatotube" can be considered to be a conventional rectangular TLC plate bent around until its edges touch (an apparatus for accomplishing almost this very act has been described; it serves to hold a plastic-backed TLC sheet in a spiral configuration for development).[65] The thin layer can be on either the inner or outer surface of the resulting cylinder (Figure 11). In practice, the thin layer is usually applied to the outside surface of a support such as a test tube (although precoated chromatotubes have been commercially available). Sample application is accomplished either by using a syringe or by immersing the lower end of the tube into a shallow reservoir of sample solution. This is followed by one or more predevelopments (short) with a polar solvent to concentrate the sample into a tight band before development. Alternatively, the sample has been applied with a spray head generating a narrow pattern of fine mist.[66] This method is only applicable to tubes coated on the outer surface. It has the advantage of producing a narrow band without the necessity for tedious predevelopment.

A modification of tube TLC uses a test tube

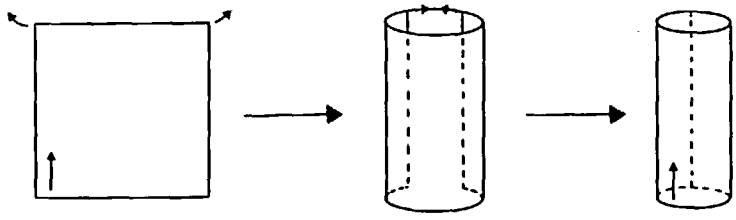

FIGURE 11. Cylindrical TLC can be considered a version of conventional TLC in which the plate is bent around into a cylindrical shape. Solvent migration (light arrow) is parallel to the cylinder axis. The layer may be coated either on the inner or the outer surface of the layer.

with a small hole in the bottom as a backing.[67] The tube is coated by dipping in a slurry of the sorbent used. The sample solution is introduced to the inside of the tube, from which it flows through the hole and is pulled into the layer by capillarity. Following sample application, the tube is filled with the developing solvent which penetrates the layer in the same manner. Development of TLC tubes is normally carried out merely by standing the cylinder on end in a conventional N-chamber. However, when test tubes are used as a backing, they must be suspended either within an N-chamber or within a larger concentric tube during development.[61]

Cylindrical TLC with the thin layer coated on the inner surface of the cylinder has been used in systems which facilitate quantitation with a GC-type flame ionization detector (FID).[68,69] After development, these tubes are connected to an apparatus which allows carrier gas to flow through the tube while a heated zone is gradually passed along the length of the outer surface. Organic samples are vaporized or pyrolized and are swept by the carrier gas into the FID for quantitation. The concept of cylindrical TLC can be extended to cover a discussion of "stick TLC" and "chromatosticks." The former are essentially chromatotubes with diameters that are sufficiently small that they are more conveniently based on a solid rod than on a hollow cylinder. In contrast to most cylindrical TLC techniques, which have generally been aimed at preparative work, stick TLC has been applied primarily to analytical separations. It allows a limitation on horizontal spot spreading (and hence improved sensitivity) without introducing the "edge effects" which often limit resolution when channeled conventional TLC plates are used for the same purpose.[70] Glass rods with a thin layer permanently bonded to the surface by sintering with powdered glass have also been described.[71] These provide a durable, organic binder-free medium which can be used with a specially modified FID for quantitative analysis. However, the performance of sintered rods (or plates) described in the literature has generally been found to be somewhat inferior to that of conventional layers.[72] This can be ascribed to the increase in effective particle diameter resulting from the sintering process. Chromatosticks[73] represent an attempt to combine the loading capacity of columns with TLC development conditions. They are made by packing a paper tube with a binder-containing adsorbent slurry. After the binder has set, the paper tube is peeled away and the resulting rod is activated by heating. Development is carried out by standing the rod on end in an N-chamber. After development, the column is sliced and the sample components are eluted from the adsorbent.

There is no a priori reason why the performance of cylindrical TLC cannot approach or equal that of conventional TLC. There is also no reason why the performance of cylindrical TLC should exceed the performance of conventional TLC. The major application of cylindrical TLC has been in preparative work, for which the cylindrical format greatly eases the problems associated with plate handling and sample recovery. The sorbent zones containing the sample components of interest easily can be removed from the backing by rotating the cylinder against a spatula blade held over the opening of a receiving vessel.[61] This approach minimizes possible cross-contamination of sample zones during the recovery process. Preparative ("thick") layers have also been coated onto conical surfaces (Figure 12) such as funnels.[74,75] Such layers have found application

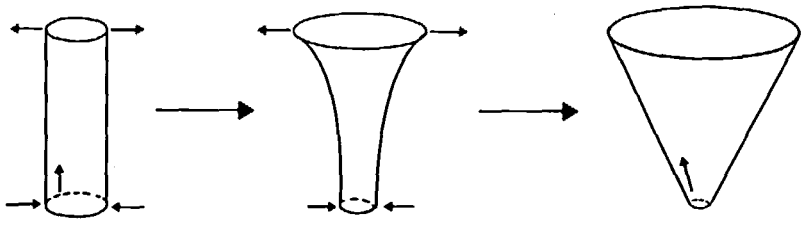

FIGURE 12. Conical TLC can be considered a distortion of cylindrical TLC. The distortion gives rise to a velocity gradient in the bed during development. Sample zones are concentrated as a result.

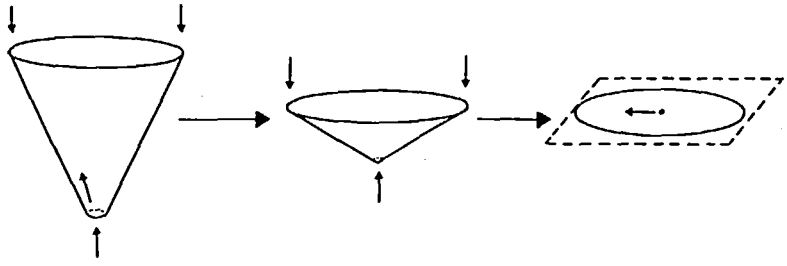

FIGURE 13. Radial TLC can be considered a distortion of conical TLC. The result is a clearly defined (hyperbolic) solvent velocity gradient which effectively reconcentrates sample zones.

in preparative TLC because of the following two advantages:

1. As development progresses, the horizontal size of sample zones increases. In effect, this decreases the loading and thus increases the apparent efficiency in many cases.

2. A solvent velocity gradient is formed. Since the solvent must expand into a steadily increasing volume as it travels through the layer, its velocity decreases from the reservoir to the solvent front (this is in contrast to the situation in conventional TLC, where solvent velocity at any given time is constant at all points on the plate). The existence of this negative velocity gradient means that the trailing edge of a sample zone experiences a higher mobile-phase velocity than does the leading edge. This effect counteracts zone broadening and results in narrower spots.

The degree of reconcentration depends on the steepness of the gradient. This, in turn, depends on the angle between the cone surface and the vertical axis. As this angle is decreased until it approaches zero, the steepness of the gradient decreases until, in the limiting case of cylindrical TLC, no velocity gradient is produced. On the other hand, if the angle is increased until it approaches the perpendicular, the gradient steepens to that described by radial chromatography.

In radial chromatography,[4,76-79,117] the solvent is admitted to a central point on a horizontal TLC plate and migrates radially, forming a circular wetted zone of gradually increasing area (Figure 13). The flow of solvent is limited by the delivery of solvent to the center of the plate. Because this flow must expand into a volume which increases as a function of the square of the distance from the center, the solvent velocity decreases according to the equation[80]

$$v = \bar{K}/X^2 \qquad (36)$$

Because the solvent velocity at any distance from the origin is less than the limiting velocity in a capillary system, the velocity at any point on the plate is constant with time. The existence of the velocity gradient gives rise to the following relationship between relative migration velocity (R) and relative migration distance (R_f):

$$R_f = \sqrt{R} \qquad (37)$$

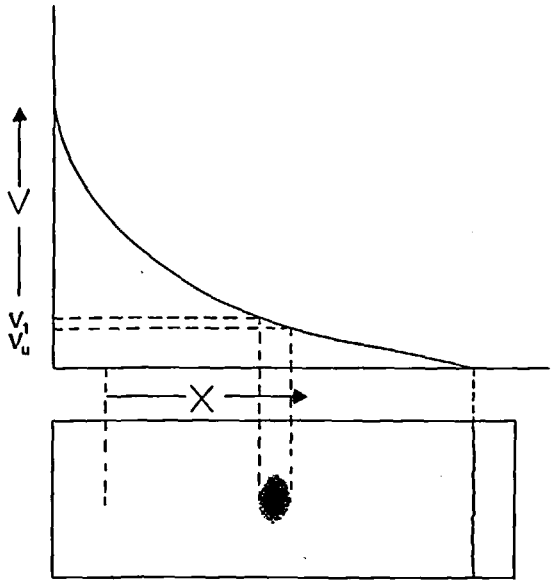

FIGURE 14. Zone concentration by a velocity gradient. The lower edge of the spot is carried at a higher velocity than the upper edge. This effect counteracts zone broadening and results in a narrowing of the spot.

Evaluation of the observed plate height in radial TLC according to Equation 20 for the velocity profile in Equation 36 yields

$$\bar{H} = BRX^2/3\bar{K} + (AC\bar{K}/X^2)\arctan(\sqrt{AX^2/C\bar{K}}) - A(X_o/X) \quad (38)$$

If $\bar{K}$ is assumed to be numerically equal to the pseudodiffusion coefficient for conventional TLC solvent flow, then Equation 38 predicts observed plate heights comparable to those in conventional TLC.

However, Equation 20 does not take into account the reconcentration of spots brought about by the velocity gradient. The effect of this gradient is proportional to the relative difference in velocity between the leading and the trailing edge of the spot (Figure 14).

$$dw/dX = R(\Delta v/v) \quad (39)$$

The mathematical treatment is greatly simplified (at the expense of only minor inaccuracy) if we assume the velocity of the spot to equal the velocity of the upper edge.

$$dw/dX = (RX^2/\bar{K})(1/X^2) - [1/(X - w)^2] \quad (40)$$

On the other hand, the degree of spot broadening per unit distance is given by

$$dw/dX = 2\sqrt{H/X} \quad (41)$$

If we presume a steady state at which reconcentration and broadening are balanced, these effects must be equal and opposite in sign; the resulting equation can be solved for the steady state spot width.

$$w = X(1 - [1/(\sqrt{1 + [\sqrt{4H/R^2X}]})]) \quad (42)$$

An apparent plate height (H) can then be calculated from the equation

$$(H) = w^2/16X \quad (43)$$

If we evaluate the apparent plate height using values of H = 0.001 cm, R = 0.5, and X = 2.5 cm, we obtain an apparent plate height of 0.00022 cm. The reconcentration of sample zones in radial chromatography allows the generation of bands which are narrower than those produced by a conventional TLC system of the same intrinsic efficiency. This band narrowing invariably results in higher sensitivity; however, its effects on resolution must be judged by comparison with the change in separation produced by the solvent velocity gradient. For example, if we consider two spots whose relative migration velocities are 0.48 and 0.51 ($r \cong 1.04$) in a radial TLC system whose solvent migration distance is 3.5 cm, we would predict a center-to-center separation (ΔX) of

$$\Delta X = (\sqrt{R_2} - \sqrt{R_1})X_s \cong 7.45 \times 10^{-2}\ \text{cm} \quad (44)$$

We can also calculate the average width of these spots assuming a plate height of 0.001 cm as 9.44×10^{-2} cm, implying a resolution of approximately 0.52. The equivalent plate height is

$$\bar{H}_e = X(r - 1)^2/16R_s^2 \cong 3.9 \times 10^{-4}\ \text{cm}$$

This is an improvement in performance of a factor of approximately 2.5.

Acceleration of the solvent flow in radial chromatography does not, to a first approximation, alter the shape of the solvent profile; the effect is merely that of an increase in the solvent velocity parameter $\bar{K}$. However, it should be pointed out that the decrease in efficiency which occurs at higher than optimum values of $\bar{K}$ is more

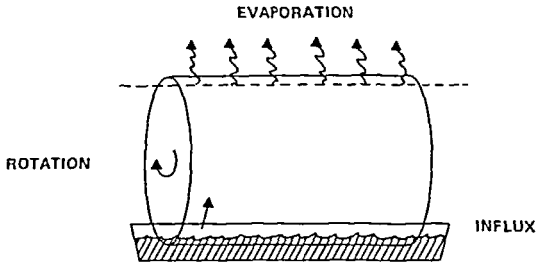

FIGURE 15. Drum TLC. The cylinder (with the thin layer on the outer surface) is rotated opposite to the direction of migration at the migration velocity of the spots of interest. As a result, the spots are maintained between the reservoir and the evaporative front as they migrate over a long expanse of thin-layer bed.

than compensated for by the decrease in migration time. Thus, in effect, accelerated chromatography trades efficiency for speed. The object is not performance measured in theoretical plates, but rather in delivery of theoretical plates per second.

The idea of increasing solvent velocity by rapidly spinning the substrate originated in attempts to speed up the extremely slow process of PC.[81-83] It was plagued by uneven solvent flow and by problems with the necessarily complex apparatus.[84] With the advent of the comparatively rapid process of TLC in the early 1960s, centrifugally accelerated methods became considerably less popular, although adaptations to TLC have been made.[85]

Improvement in delivery is also the object of "drum TLC."[53] The apparatus can be visualized as comprising a TLC plate bent backward until the top and bottom edges meet to form a cylinder. Development is perpendicular to the axis of the cylinder, with provision for evaporating solvent from the upper portion of the drum. Once continuous development has been established (see below), the drum is rotated in a direction opposite that of solvent migration at a sufficient rate to maintain the sample components of interest in the working region of the bed (Figure 15). The result is a bed of arbitrary length (limited only by operator patience and by the spreading of the spots) which can, therefore, generate an arbitrarily large theoretical plate number. The advantage of drum TLC is that it can mimic the separation of a very long bed without encountering the increased plate height (the result of lower than optimum solvent velocity associated with long beds in conventional TLC).[86] This advantage is partially offset by the experimental difficulty of choosing a drum rotation speed for often invisible components of interest.

Continuous Development TLC Techniques

Continuous development TLC systems are defined as those systems in which the solvent is evaporated from the plate. The object is the attainment of a steady state in which solvent influx into the thin layer is exactly balanced by solvent evaporation from the plate. This steady state can be maintained for an arbitrary length of time, during which sample components are carried forward from the origin to eventual deposition at the solvent front. Components which have not yet completed this journey are found in the bed at the termination of development. In general, these components have much lower relative migration velocities than those associated with conventional TLC (usually, R values in continuous development are below 0.1). We will confine our discussion of continuous development to its effect on such slow-moving spots.

Two fundamental types of continuous development can be distinguished. In the first type, typified by the system of Truter,[87,88] evaporation is confined to the upper edge of the TLC plate (Figure 16). The major part of the layer is in contact with a saturated atmosphere and, hence, experiences little or no net evaporation. This type of continuous development may be considered to occur in two stages. The preliminary stage consists of a conventional TLC development. The solvent front has not yet reached the zone of evaporation. The effect of this stage on the low-velocity spots under consideration is negligible; for practical purposes, they may be considered to have remained at the origin. However, once the solvent front has reached the zone of evaporation, a relatively abrupt transition to steady-state continuous development occurs. Because the solvent velocity is constant (determined by the distance between the reservoir and the front, $v = K/X_s$), the observed plate height is given by the coupled plate height equation.

$$\bar{H} = BRX_s/K + 1/(1/A + X_s/CK) \quad (45)$$

Evaluation of this equation for the same optimum parameters used to describe conventional TLC (except for R, which we have changed from 0.5 to

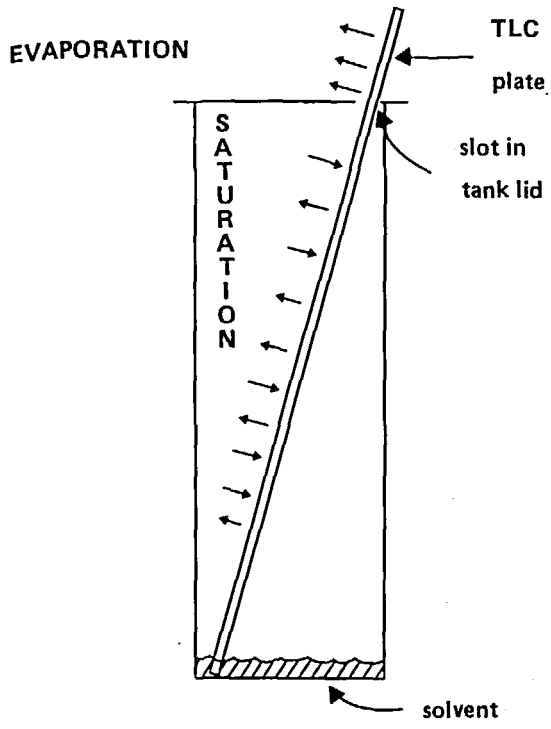

FIGURE 16., Continuous-development TLC. Evaporation is limited to the upper edge of the plate. Once a steady state has been established, the solvent flows through the bed at a constant velocity.

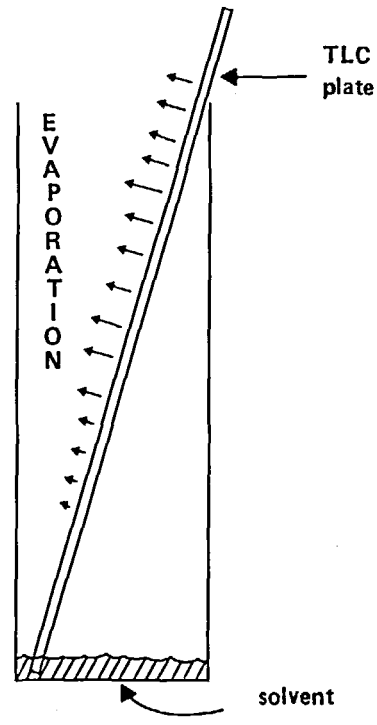

FIGURE 17. Evaporative TLC. Evaporation occurs over the entire wetted plate surface. As a result, a velocity gradient is formed on the plate.

0.05) suggests a plate height on the order of 1×10^{-3} cm. Thus, the use of this type of continuous development does not increase efficiency. The primary advantage is that it allows a reasonable migration distance for low-velocity components (albeit at the cost of deteriorated separation of faster components). This, in turn, is useful because of the observation that selectivity in TLC (defined here as the ratio of relative migration velocities) increases as the average relative migration velocity of the components of interest decreases. This is equivalent to saying that, in general, the difference in relative migration velocities remains constant. Improved resolution of difficult pairs can often be obtained by decreasing the solvent strength while turning to continuous development to exploit this effect.

The second type of continuous development has been called "evaporative TLC."[90,91] In evaporative TLC, evaporation of the solvent occurs over the entire area of the TLC plate and during the entire course of the development (Figure 17). Thus, the continuous development steady state may be considered as a limiting case in which the solvent front has advanced to a position which allows sufficient wetted area to evaporate all of the solvent which enters the bed. The result must be a solvent velocity profile which generally decreases from the reservoir to the solvent front. A complete description of the effect of evaporation in TLC has been provided by Stewart and Wendel.[91] We will continue the approximation used above and consider only the effect of the steady state condition on slow-moving spots. The salient feature of Stewart's treatment of this aspect is the observation that, although the solvent velocity profile in evaporative TLC is complex, its effects are largely counteracted by corresponding changes in solvent concentration on the TLC plate. The net effect is that spot velocity as a function of distance from the reservoir can, to a first approximation, be described as a straight line.

We have already noted that the effect of a negative solvent velocity gradient (decreasing from the reservoir to the front) is a decrease in both zone width and separation between zones. The

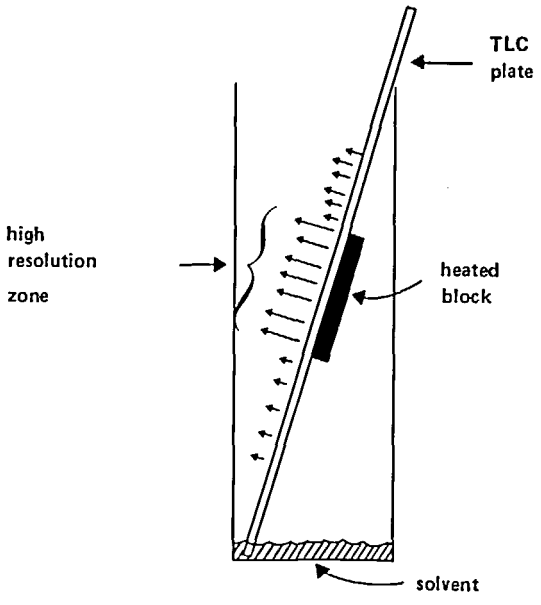

FIGURE 18. Hot-plate TLC. A heated block is used to accelerate evaporation from one region of the plate. The solvent velocity gradient in this high-resolution zone is accentuated; spots moving through the zone become highly concentrated.

effect of the gradient on resolution (and hence on the equivalent plate height) depends on the relative magnitudes of these two effects.

We can use a procedure similiar to that given for radial TLC to calculate the steady-state zone width (the width at which broadening and the gradient reconcentration are balanced) as

$$w = 2(X_s - X)\sqrt{H/X} \quad (46)$$

The separation between zones in evaporative TLC is a function of the migration time as well as of the position of the origin, the distance from the reservoir to the solvent front, and the relative migration velocities of the sample components. If we assume evaporation to occur only between the origin and the solvent front, then the position (distance from the reservoir) of a spot after migration time (t) is given approximately by the equation

$$X = X_s - (X_s - X_o)\exp[-RKt/X_o(X_s - X_o)] \quad (47)$$

The predicted behavior of sample components of relative migration velocities 0.05 and 0.04 after 1-hr migration time with the solvent front 5 cm from the reservoir and a plate height of 0.001 cm indicates a separation of ≅0.35 cm and an average spot width of ≅0.08 cm. This represents a resolution of ≅4.1, which translates to an equivalent plate height of 6.5×10^{-4} cm. These results indicate that, in principle, evaporative TLC can provide an increase in performance over that of conventional TLC separations. Thus, evaporative TLC is capable not only of exploiting the increased selectivity associated with the use of nonpolar solvents and resulting low relative migration velocities, but also of providing improved performance over conventional TLC. However, the resulting improved resolution of slow spots may be obtained at the expense of decreased resolution of faster moving components.

The advantages of evaporative TLC have been exploited in a technique called hot-plate chromatography,[92,93] in which solvent evaporation is achieved by applying heat to the back of the TLC plate (Figure 18). One variation suggested for hot-plate chromatography is the use of a relatively narrow heated high-resolution zone[93] in which evaporation occurs. Spots which traverse this zone become highly concentrated, with attendant improvements in sensitivity of detection of trace components.

Evaporative TLC can also be carried out in the commercially available BN-chamber, a horizontal apparatus which allows control of atmosphere composition and plate temperature.[59] The formation of a negative solvent velocity gradient on the plate may also be responsible for the improvement in resolution ascribed to the use of sandwich chambers rather than normal chambers for conventional TLC development.[99] The gradient is the result of net solvent evaporation from the plate surface into the unsaturated atmosphere during development.[91]

The preceding discussion implicitly assumes that the solvent system used is homogeneous — that it behaves as a single component. In practice, this assumption is rarely true in TLC; this is one of the reasons that correlations between thin-layer systems and column systems have been notoriously difficult to obtain. Although the effects are interrelated, it is useful to distinguish between actual demixing (the formation of a solvent composition gradient on a TLC plate as a result of selective retardation of the more polar solvent components by the layer) and the effects of the chamber atmosphere composition on plate activity and selectivity.

With solvents of two or more components,

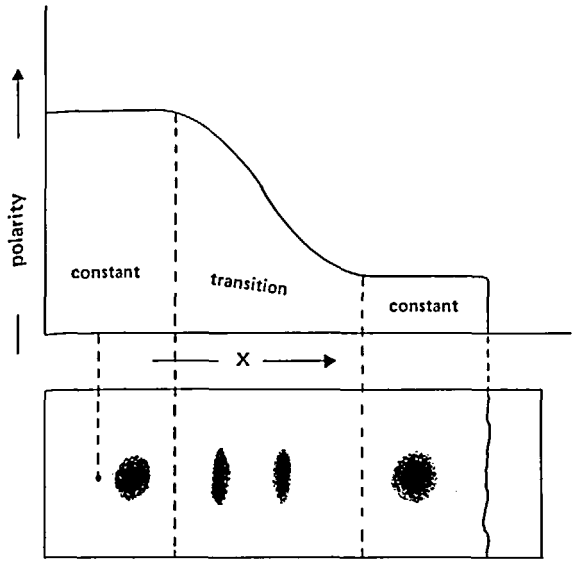

FIGURE 19. Solvent demixing during development gives rise to transition regions in which solvent polarity gradients exist. Zone concentration occurs in these regions.

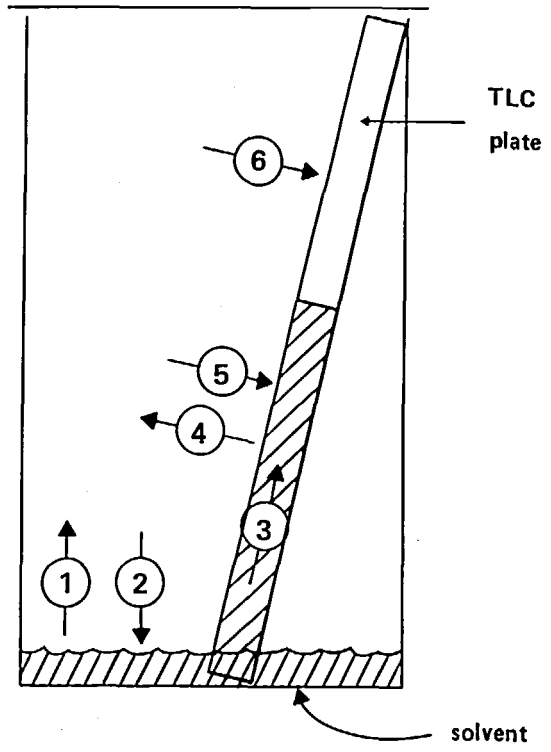

FIGURE 20. Solvent transfer in TLC occurs by a variety of mechanisms. (1 and 2) Evaporation and condensation from the liquid solvent reservoir results in changes in mixed solvent composition based on differences in equilibrium vapor pressure. (3) Migration of solvent through the plate; this results in selective depletion of the more polar component in the advancing solvent. (4 and 5) Evaporation and condensation from the wetted surface of the TLC plate. (6) Condensation on the dry surface of the plate. Condensation of polar solvent components on the upper portion of the plate minimizes the effect of solvent demixing during migration through the bed.

solvent demixing occurs as the result of selective sorption of the more polar component by the layer.[95] The result is that the more polar component of the leading zone of the moving solvent becomes depleted. The effect is more pronounced when the solvent components differ greatly in polarity. The result of demixing is that regions of differing polarity are created in the moving solvent. These may be described approximately as constant polarity regions separated by transition regions (Figure 19). The transition regions incorporate a gradient of solvent polarity (see below) in which steepness varies with the polarity difference between the solvent components. The steepness of the gradients in the transition regions also depends on the degree of saturation of the chamber atmosphere; the vapor phase provides a mechanism for the transfer of polar component to the upper section of the plate (Figure 20).

DeZeeuw et al.[96] have investigated the influence of vapor composition on selectivity. Viricel et al.[95] have noted that demixing is more difficult to observe in saturated tanks, because vapor-phase transfer tends to make polarity gradients less steep. The general consensus of opinion is that solvent polarity gradients tend to be steeper in unsaturated than in saturated chambers. Thus, care must be taken in adapting an N-chamber solvent system to an S-chamber and vice versa.

The "edge effect," an occasional disadvantage of sandwich-chamber TLC, is the result of uneven saturation of the atmosphere with solvent vapors. The symptoms, uneven secondary solvent fronts and R_f values across the plate, seem to be especially prevalent when chloroform/alcohol solvent systems are used. It has been suggested that the cause of the problem is turbulent mixing due to differences in density between the solvent component vapors. A decrease in spacer thickness has been observed to ameliorate the problem.[96]

C. Gradient TLC Techniques

We have discussed the effects of solvent

velocity gradients on the performance of radial and evaporative TLC. We will briefly consider the effect of solvent and stationary phase gradients on performance in conventional TLC.

In GC and LC, the conventional wisdom maintains that the purpose of gradients (thermal for GC, solvent strength for LC) is the facilitation of the separation of sample mixtures covering a wide range of k' values under constant conditions.[97] For example, the typical case quoted in GC considers the analysis of a mixture containing both high- and low-boiling components. Optimum conditions for the latter destroy resolution of the former and vice versa. In this case, the solution is a change in conditions during the course of the separations. Thus, the low-boiling materials are eluted at their optimum, and the high-boiling materials are then eluted at their different optimum. An important feature of most treatments of this process is the assumption that, at any given instant, identical conditions exist over the entire length of the bed. This assumption implies that both the leading and the trailing edges of a given peak "see" the same conditions; as a result, no reconcentration can take place. Resolution under programed temperature or solvent conditions can then be no better than the resolution of the same components under isothermal or isocratic conditions. This assumption is certainly valid in GC, in which temperature changes affect the column as a unit. It is also arguably true in LC, in which gradients are shallow and zone widths small as compared to the column length.

The situation in TLC is more complex, because a variety of continuous or stepwise gradients can readily be generated. Such gradients can also vary greatly in steepness relative to spot dimensions. However, we can intuitively classify TLC gradients into one of the following two groups:

1. Gradients with no inflection point. These can be considered analogous to the linear and hyperbolic velocity gradients discussed above in connection with evaporative and radial TLC. We can make the following generalization: When no inflection point exists in the profile, the width of at least one of the spots will be decreased to a greater extent than the center-to-center separation between spots is decreased (Figure 21). Such gradients are thus capable of improving TLC performance (decreasing the equivalent plate height of a given system). However, there is a built-in limitation to the effectiveness of TLC gradient methods; as spot widths decrease, the effective sample loading in the layer increases. The resulting nonlinearity of distribution isotherms eventually decreases efficiency to the point at which no further net reconcentration can occur.

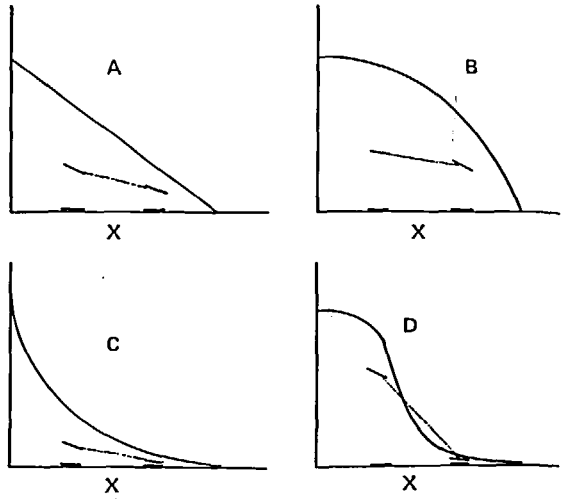

FIGURE 21. TLC performance (described as the equivalent plate number or equivalent plate height) is improved when spot concentration is more significant than the reduction in separation between spots. This condition is met if the gradient effective across a single spot (the intraspot gradient) is steeper than the gradient effective between adjacent spots (the interspot gradient). When the velocity gradient is uninflected (A, linear; B, convex; C, concave), at least one intraspot gradient (solid lines) must be steeper than the interspot gradient (dotted lines). This is not necessarily true of inflected gradients (D).

2. Gradients with an inflection point. The width of both spots may be decreased to a lesser extent than the center-to-center separation between spots. Whether this occurs depends on the location of the inflection point with respect to the spots, the steepness of the gradient, and the relative migration velocity difference between the sample components (Figure 21).

A number of gradient TLC techniques have been defined.

Polyzonal TLC[98] in unsaturated (e.g., sandwich) chambers – This technique exploits the solvent polarity gradient formed as a result of demixing (see above). Spots migrating in regions of constant polarity are not reconcentrated except incidentally as the result of solvent velocity gradients produced by evaporation in the sandwich chamber. Similarly, pairs of spots migrating in the same region experience no decrease in separation. However, the separation between spots in different

regions is greatly reduced (in fact, this is the usual purpose of polyzonal TLC; it allows the separation of mixtures whose components vary widely in polarity on a single plate). Spots migrating in a transition region become highly reconcentrated because gradients in sandwich chambers are usually steep. However, separation between adjacent spots in such a region is typically reduced to near zero.

Polyzonal TLC in saturated (e.g., N-) chambers – Spots migrating in regions of constant polarity are not reconcentrated, nor (if they are in the same region) is the separation between them decreased. Spots in different regions are brought closer together. Since the solvent velocity profile in a transition region must have an inflection point, we can assume that, to a first approximation, the decrease in separation between adjacent spots is greater than the decrease in spot width brought about by the gradient. Resolution between spots in a transition region will therefore be diminished. (An exception to this statement occurs for very shallow gradients on which sizable regions are approximately linear.)

Continuous solvent gradients, continuous adsorbent gradients – The effect of continuous gradients on performance depends on the exact profile of the gradient (see the above discussion).

A large number of techniques which utilize the formation of gradients in TLC have been described. These gradients may be formed in the solvent reservoir, in the thin layer, or by the influence of the atmosphere above the layer. If properly chosen, such gradients can increase performance. More typically, however, the use of gradient techniques in TLC leads to decreased efficiency. Gradient TLC (as well as gradient GC and LC) is useful primarily in the analysis of mixtures in which the components vary widely in chromatographic properties. The primary objective in these cases is the trading of excess separation for more convenient or faster separation.

D. Multiple-development Techniques in TLC

The term "multiple development" encompasses a variety of TLC techniques – repeated development of a TLC plate in one or two dimensions with the same or changing solvents for constant of varying distances. We will restrict our discussion of the effect of multiple development on efficiency to the case of repeated developments of a plate in the same direction with the same solvent. This restriction is not intended to dismiss the importance of two-dimensional TLC, but rather to underscore the fact that its usual purpose is increased selectivity rather than increased efficiency.

Multiple-development TLC introduces a situation which is unique in current chromatographic practice: the existence of an interface (the solvent front) which periodically traverses the stationary bed and any sample components contained within it. When the solvent front traverses a spot deposited on the thin-layer bed, the lower edge of the spot begins to move while the upper edge is still fixed in place. By the time the upper edge begins to move, the lower edge of the spot has been carried R_f times the original width (Figure 22). The result is the reconcentration of the spot to approximately $(1 - R_f)$ times its initial width.[99] Once the entire spot is in contact with the moving solvent, it begins to broaden during development, as in conventional TLC. This reconcentration occurs once on conventional TLC (mediating, to a certain extent, the ill effects of large initial spots). However, in multiple development it occurs at least once with each development. Thus, the relative importance of reconcentration in multiple development depends on the degree of broadening which occurs between reconcentrations.

The simplest type of multiple-development TLC has been called unidimensional multiple chromatography (UMC).[100,101] UMC is defined as the repeated development of a TLC plate with the same solvent in the same direction for the same distance. The technique was developed for PC in the early 1950s.[102,103] In the early 1960s a revival of interest in UMC as a tool in TLC[104,105]

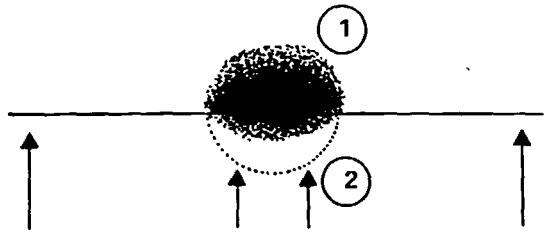

FIGURE 22. Spot reconcentration by a moving solvent front. Sample molecules ahead of the front (1) are fixed in place while molecules behind the front (2) are carried forward. The result is a reduction in spot width.

included a thorough description of its effects on center-to-center separation.[100,106] It is characteristic of work in TLC that the effect of spot reconcentration was noted, but not explicitly described at this time.

The relative migration distance of a spot after n developments ($_nR_f$) may be predicted from the equation

$$_nR_f = 1 - (1 - R)^n \quad (48)$$

When separation between adjacent spots is plotted as a function of the number of developments (n), the separation is found to increase, pass through a maximum value, then begin to decrease as successive developments are carried out (Figure 23). The number of developments producing the optimum (maximum) separation is given by

$$n_{opt} = -1/\ln(1 - R) \quad (49)$$

The maximum attainable separation ($\Delta X_{n,opt}$) is given by

$$\Delta X_{n,opt} = n(1 - R)^{n-1} \Delta R \quad (50)$$

where R is the average relative migration velocity of the compounds of interest and ΔR is the difference in the average migration velocities. The derivation of this equation assumes that R is small. Plots of the exact solution have also been presented.[107]

There is no straightforward way to estimate the spot width which results from the alternation of reconcentration and broadening. However, we have used a programmable hand calculator to model the behavior of spots during a series of UMC developments. The model is based on the assumption that the spots are reconcentrated by a factor of (1 - R) at the beginning of each development and that broadening during each development is described by

$$w = 4\sqrt{HX} \quad (51)$$

where X is the migration distance during that development. The resulting dependence of w, X, and R_s on the number of developments is shown in Figure 24 for spots of R = 0.20 and R = 0.21 (r = 1.05). Figure 24 clearly shows that resolution decreases with successive developments. The resolution at optimum separation ($R_s \cong 0.461$) represents an equivalent plate height of 1.5 X 10^{-3} cm.

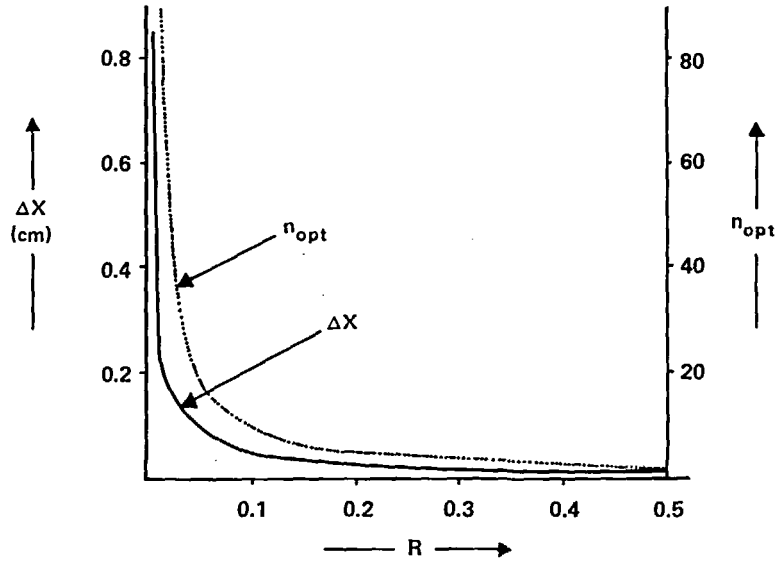

FIGURE 23. Relationship between maximum separation (ΔX) and optimum number of developments as a function of the average relative migration velocity for two spots which differ in relative migration velocity by 0.01. In principle, differences in relative migration velocity are independent of solvent strength. Thus, closely related compounds can be separated by decreasing the solvent strength and increasing the number of developments. However, such a procedure is very time consuming.

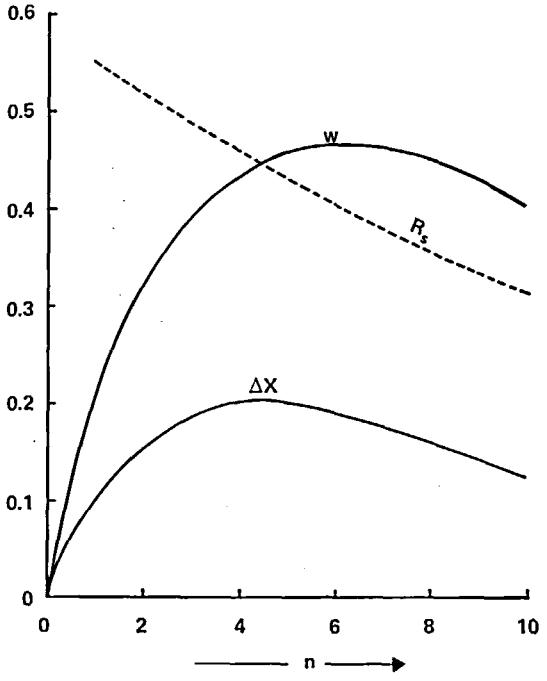

FIGURE 24. Spot behavior in unidimensional multiple chromatography (UMC); separation (ΔX, cm), spot width (w, cm), and resolution (R_s) for two spots of R = 0.20 and R = 0.21 as a function of the number of 10-cm developments (n). The initial width is assumed to be 0 cm and $\bar{H}$ is assumed to be 0.001 cm.

Thus, UMC to the first approximation decreases the efficiency of TLC. However, a somewhat different picture emerges when we consider the case of noninfinitesimal spot width (see above). Figure 25 plots resolution as a function of number of developments under the above conditions for initial spot widths ranging from 0.05 to 0.5 cm. Figure 25 clearly indicates that UMC, while decreasing efficiency in the ideal approximation of negligible initial spot width, is clearly capable of improving resolution when initial spot width is an important contribution to final spot width.

A variation on multiple development has been called incremental multiple development (IMD). IMD is defined as the repeated development of a TLC plate with the same solvent in the same direction to gradually increasing distances. IMD frequently takes the form of development for increasing fractions of the bed length (e.g., development for one fourth, one half, three fourths, and, finally, the entire bed length).[122] Like UMC, IMD decreases the performance of TLC when initial spot width is negligible. However, when initial spot width contributes significantly to final width, IMD can provide significantly better resolution than can conventional TLC (Figure 26). The advantage of IMD in such a case is the availability of a relatively large number of developments (albeit short developments) in a short period of time. A very effective spot reconcentration thus occurs early in the process. In other respects, multiple-development TLC (both UMC and IMD) shares the advantages of continuous development.[88,97,100,105] These are the result of being able to spend a sufficient length of time to move slow (low relative migration velocity) spots into the "working" section of the TLC plate. Multiple development allows exploitation of the increase in selectivity that accompanies reduction in solvent polarity. In fact, the technique of programmed multiple development (PMD) discussed below continues aspects of both evaporative and multiple-development TLC. As a result, its performance must be discussed separately from that of other types of multiple development.

E. High-performance Thin-layer Chromatography (HPTLC)

The term high performance has, within the past year, been applied to three commercial TLC systems. The first is a commercial precoated TLC plate.[3,4] The literature reports observed plate heights of 0.0012 cm and relatively short optimum solvent migration distances ($X_s \cong 5$ cm) suggest that these plates incorporate a small particle diameter (possibly ≈5 μm) and a narrow particle size distribution in the layer. The published height values suggest that such plates should be capable of generating 5000 theoretical plates in a reasonable amount of time (perhaps 15 min or less). The disadvantage of these plates is that they require extreme care in sample application (literature values suggest 10 to 100 nl) if their potential is to be realized. This poses major problems in many real-life situations which require the analysis of dilute of complex solutions for trace components. Despite the great sensitivity which results from compact spots, such low load limits appear likely to severely tax the capabilities of current detection and quantitation techniques.

The second system is a radial TLC system designed specifically for use with high-performance plates.[4] (Figure 27). The system allows controlled solvent delivery to the center of a plate mounted over an atmosphere whose

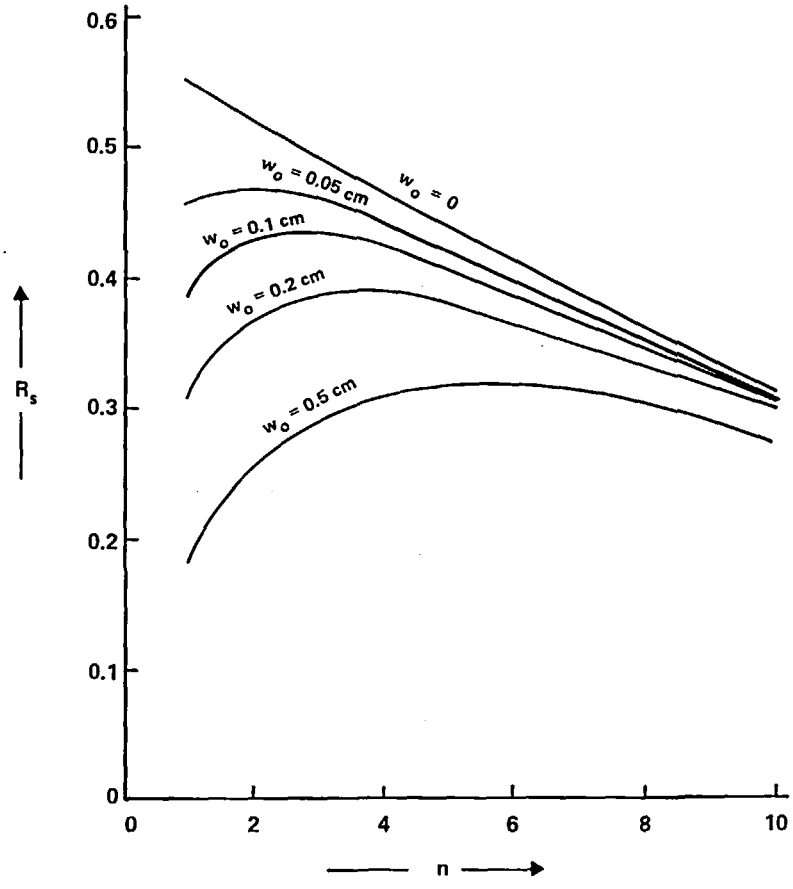

FIGURE 25. Influence of initial spot width (w_o) on spot behavior in unidimensional multiple chromatography (UMC); plot of resolution (R_s) between two spots of R = 0.20 and 0.21 as a function of the number of 10-cm developments (n).

composition can be controlled (Figure 28). Thus, this system allows the potential realization of the advantages of radial TLC, evaporative TLC, and high-performance plates in combination. On the basis of the discussion of radial TLC and evaporative TLC presented above, we would expect this system to be capable of operating at equivalent plate heights of less than 0.001 cm. The restriction on solvent migration distance with this system (typically, less than 2.5 cm) limits the equivalent plate number to less than 5000. However, separations over such a short distance are rapid; the potential delivery is in the range of two to ten equivalent plates per second. In addition to producing high efficiency, the radial HPTLC system ("U-chamber") allows control of the solvent flow rate, but not the shape of the velocity gradient, up to the maximum capacity of the bed. The composition of the vapor in the atmosphere next to the layer can also be controlled and varied during the separation to increase selectivity in specific cases.[59,108] Provision is also made for the control of plate temperature during development. Thus, the potential exists for obtaining further improvements in performance from the combination of aspects of evaporative TLC (see above) with radial TLC. The disadvantages of the system include those typically associated with radial chromatography (such as difficulties in quantitative analysis using conventional commercially available densitometers), as well as the limitation on sample loading capacity associated with the use of HPTLC plates. The former difficulty may well be temporary and trivial; it is unlikely that currently available densitometers could be used with submillimeter-wide spots without degrading resolution. The latter disadvantage is typical of high-performance chromatographic systems.

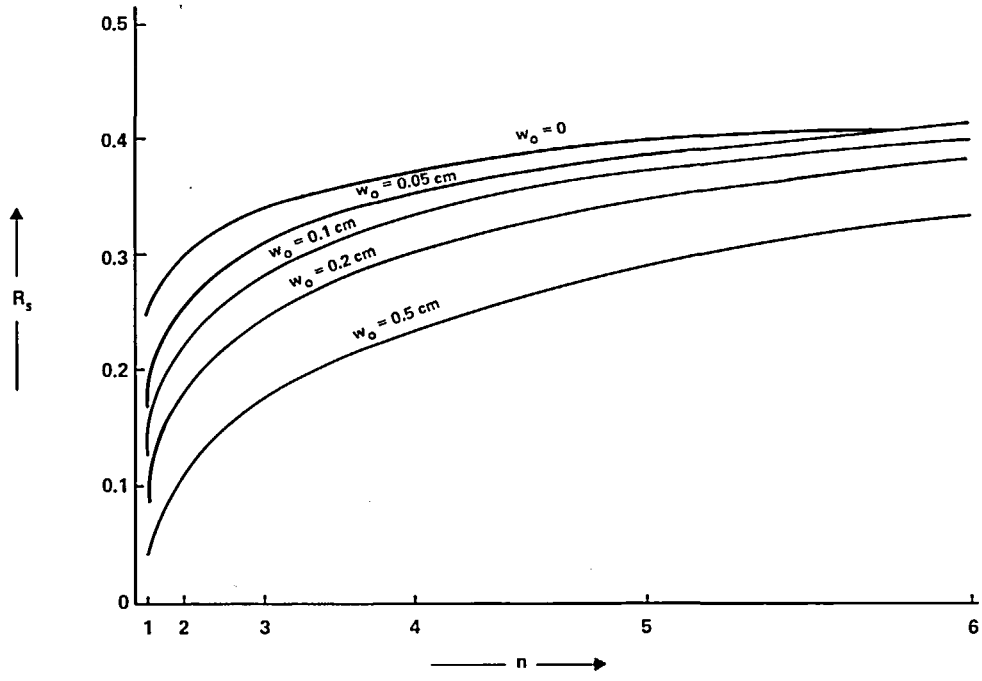

FIGURE 26. Influence of initial spot width (w_o) on spot behavior in incremental multiple development (IMD). Resolution (R_s) of two spots of R = 0.20 and 0.21 as a function of the number of developments (n); the first development is to a distance of 2 cm from the origin, the second to a distance of 4 cm, the third to a distance of 6 cm, and so on.

The third type of HPTLC is programmed multiple development (PMD).[109-111] PMD is defined as the repeated development of a TLC plate with the same solvent in the same direction for gradually increasing distances; between developments, the solvent is removed from the thin-layer bed by controlled evaporation while the plate remains in contact with the solvent reservoir. This is accomplished either by heating the back of the TLC plate or by passing a stream of inert gas across the front surface of the bed (Figure 29). In either case, the result is an increase in the rate of evaporation from the plate surface until it exceeds the rate of solvent influx through the thin-layer bed. The solvent front then recedes until it reaches a steady-state position at which the entire solvent influx is evaporated from the remaining wetted area. Thus, PMD combines features of both multiple-development (in a sandwich chamber) and evaporative TLC (during solvent removal). In addition, a further spot reconcentration occurs as the receding solvent front passes through a spot during solvent removal.[112] The result (as during solvent advance) is a decrease in spot width because the trailing edge of the spot is swept forward for a time while the leading edge is fixed in place. PMD instrumentation (Figure 30) allows a wide variety of development programs to be performed. A summary of typical program parameters is given in Table 2.

A variation on the PMD principle uses selective evaporation from a narrow vertical zone of the thin-layer bed during solvent removal[114] (Figure 31). The result is a solvent flow from both sides of the bed toward the center line of this zone, perpendicular to the direction of solvent migration. This flow serves to reconcentrate spots laterally and results in dramatically increased sensitivity as compared to conventional TLC.

The wide variety of possible PMD programs makes the formulation of explicit relationships between PMD parameters and performance difficult. However, apparent plate numbers have been shown to increase with increasing numbers of developments. Apparent plate numbers in excess of 100,000 have been reported for a program of 68 developments lasting 72 hr.[113] Although only crude models for PMD spot behavior presently exist, we can make a rough prediction of performance by combining alternate periods of incre-

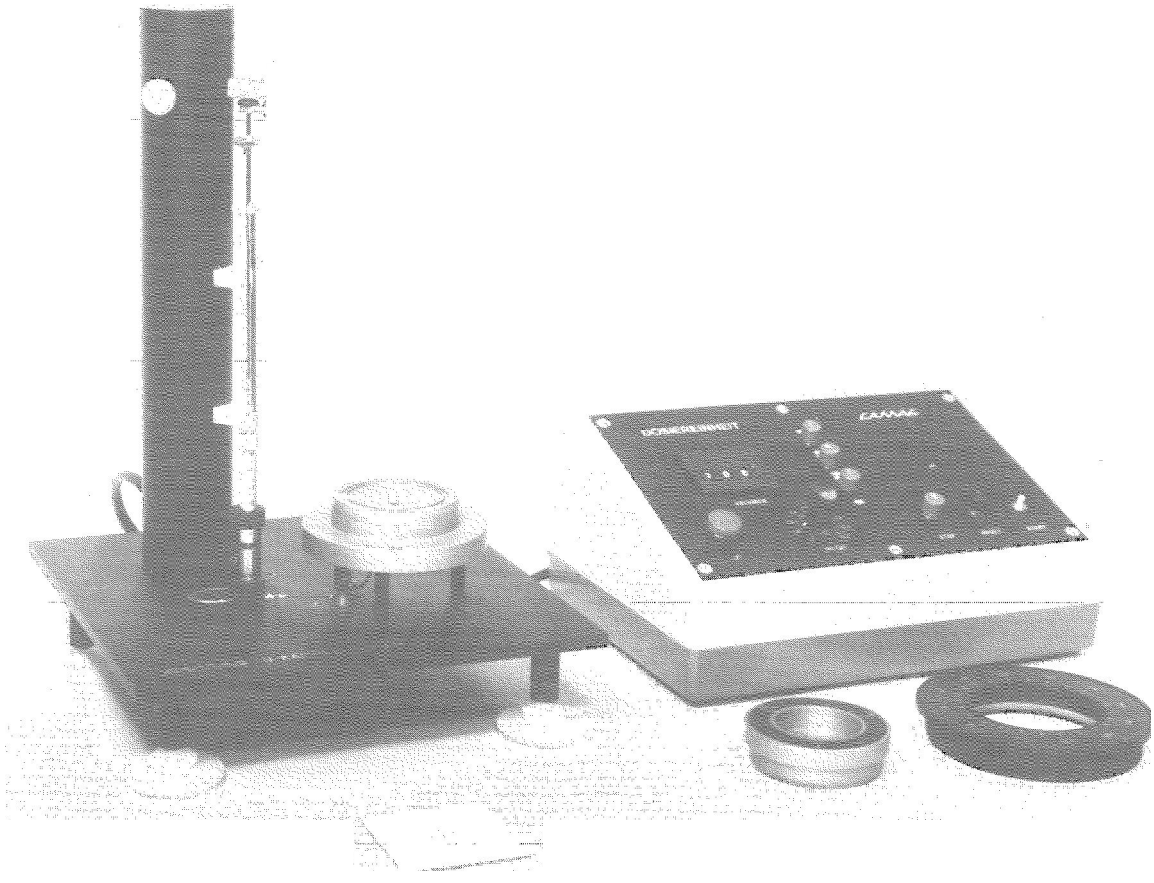

FIGURE 27. CAMAG U-Kammer apparatus for radial high-performance thin-layer chromatography. (Courtesy of CAMAG Inc., New Berlin, Wis.)

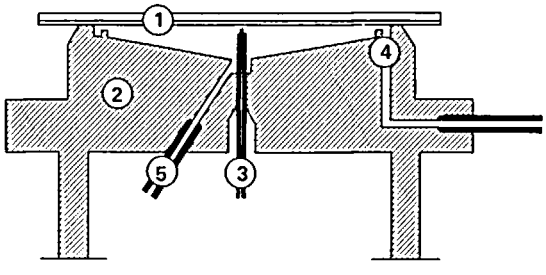

FIGURE 28. Cross-sectional diagram of CAMAG U-Kammer. The HPTLC plate (1), measuring 50 X 50 mm, rests with its layer facing downward on the U-chamber body. (2) Elution solvent is fed to the center of the plate via a platinum-iridium capillary (3) of 0.2 mm internal diameter. Vapor phase, made up externally, may be passed through the chamber, in through the circular channel (4) and out through the center bore (5) before, during, and after chromatographic development. The direction of gas flow may also be reversed. (Courtesy of CAMAG Inc., New Berlin, Wis.)

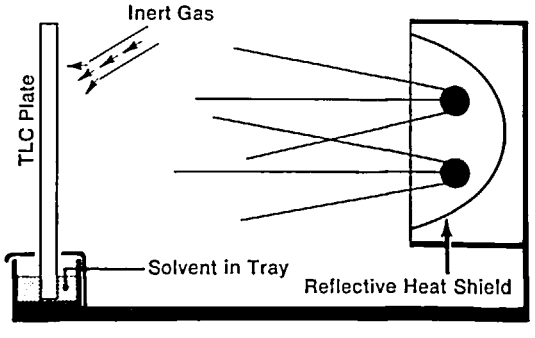

FIGURE 29. Schematic diagram of PMD developer. The thin-layer plate is approximately 5 in. distant from and centered with respect to the radiator. (Courtesy of Regis Chemical Company, Morton Grove, Ill.)

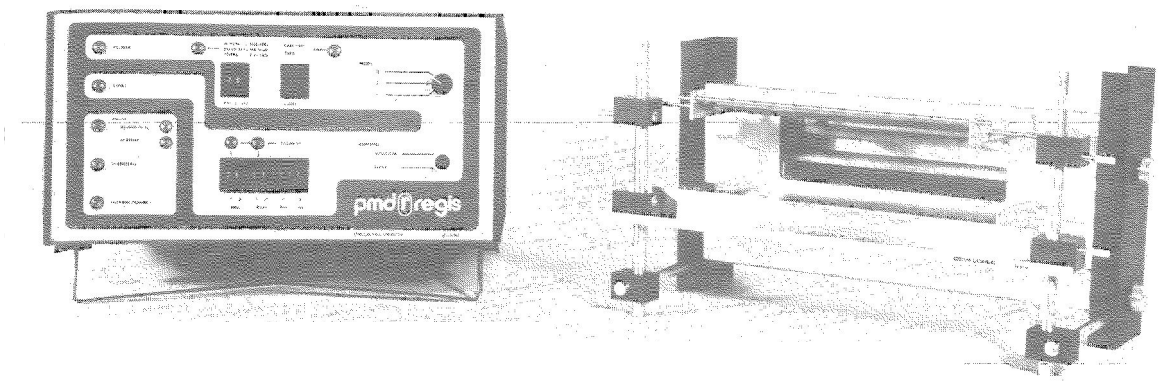

FIGURE 30. Programmer and developer for programmed multiple development (PMD). (Courtesy of Regis Chemical Company, Morton Grove, IIL)

TABLE 2

PMD Program Parameters

Parameter	Symbol	Limits	Function
Cycles	n	1–99	Sets the number of developments and dryings in the program
Mode	mode	1, 2, or 3	Establishes the dependence of development time (T_n) on cycle number (n), mode 1, $T_n \propto n$; mode 2, $T_n \propto n(n+1)/2$; mode 3, $T_n \propto n^2$
Solvent removal	F or S	–	F, fixed (constant) drying time between developments; S, scheduled (proportional to development time) drying time between developments
Advance time	t_a	10–100 sec	Sets time for first development; subsequent development times are multiples of t_a
Advance power	P_a	0–12.5%	Sets the power level of the IR heaters (100% = 400 W) during development
Removal time	t_r	0–100 sec	Sets time for drying after the first development; drying time after subsequent developments is either equal to fixed (F) or a multiple of t_r scheduled (S)
Removal power	P_r	0–100% (F) or 0–50% (S)	Sets power level of the IR heaters during drying
Preheat time	t_{pr}	0–100 sec	Sets a preconditioning time before the first development, during which the heater is controlled by the removal power level.
Interim power	P_{int}	0–100%	Sets the power level of the IR heaters before, after, and during pauses in the program

Note: These parameters are entered into the PMD programmer by means of thumbwheel switches on the front panel. They determine the sequence of development and drying which constitutes a PMD program.

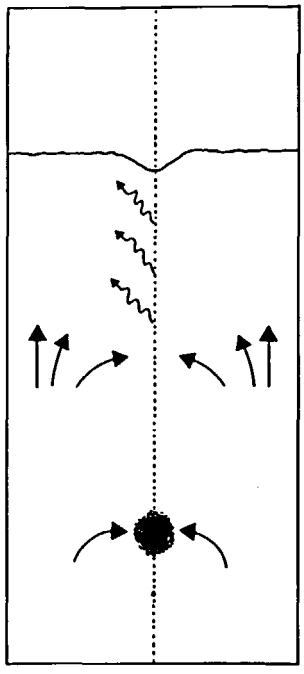

FIGURE 31. Centered PMD. Selective evaporation along the chromatogram (vertical) center line, accomplished by heat or a nitrogen stream, induces horizontal solvent flow toward the center of the bed. This flow concentrates spots and counters horizontal spreading.

mental multiple development (see above) and evaporative TLC with a receding solvent front.[118] Results for various initial spot widths from such a model are shown in Figure 32. The optimum resolution predicted corresponds to an equivalent plate height of 0.0007 cm. Figure 32 suggests and published data[119] support the belief that the advantages of PMD over conventional TLC become more pronounced as sample loading and initial spot size are increased; this feature is shared with other forms of multiple development. In addition, PMD shares with multiple development the independence of final spot position from origin location.[119-121] The mechanism of this spot alignment has been demonstrated to be the same as the spot reconcentration mechanism discussed above.[120] The theory and practice of PMD have been reviewed.[113,123]

V. SUMMARY AND CONCLUSIONS

We have chosen to limit our discussion of HPTLC to a strict definition of performance as defined by the efficiency of the chromatographic system. By analogy with current practice in gas and liquid chromatography, we have defined "high performance" in TLC as the capability of generating the equivalent of 5000 or more theoretical plates in a reasonable length of time or of delivering the equivalent of one or more plates per second.

Application to TLC of the coupled plate-height equation suggests that well-made plates should be capable of plate heights in the micrometer (0.0001 cm) range; the best current commercial precoated TLC plates approach this, with reported values of 0.0012 cm. Such plates are capable of high performance by the above definition. In addition, however, a number of TLC techniques which give rise to solvent velocity gradients are capable of increasing the resolution available from a given system. Typical improvements from such techniques are expected to result in a doubling of the equivalent plate number.

HPTLC systems allow the combination of the unique advantages of TLC and yield sensitivity and resolution fully comparable to those of GC or HPLC.

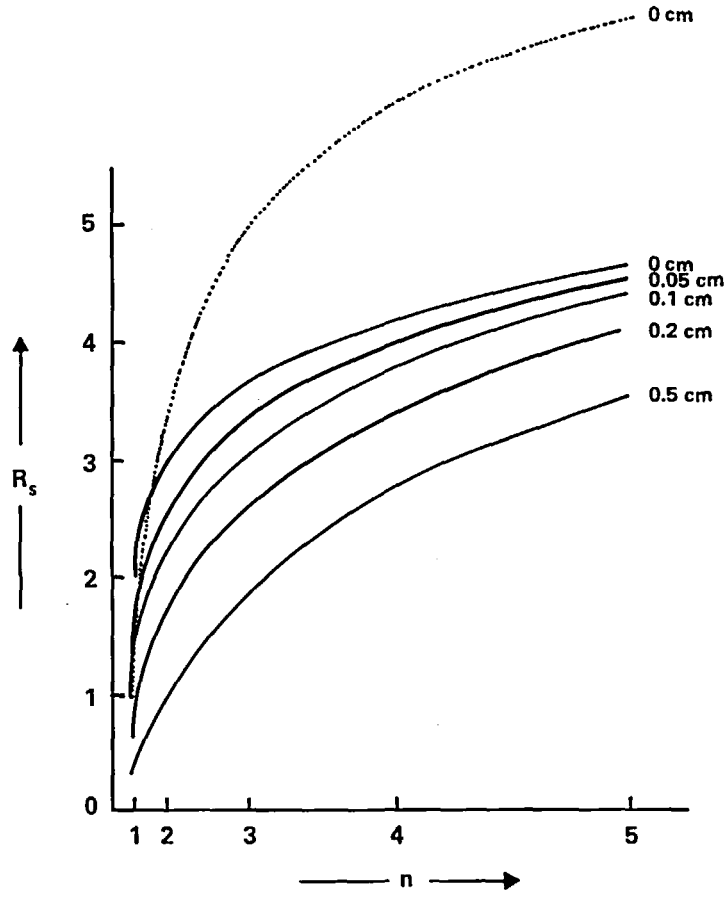

FIGURE 32. Influence of initial spot width (w_o) on spot behavior in programmed multiple development (PMD); resolution (R_s) between two spots of R = 0.20 and 0.21 as a function of the number of developments (n). The development program follows the pattern of IMD (see Figure 26). Heavy emphasis on PMD solvent removal (dotted line) and light emphasis on PMD solvent removal (solid lines) are assumed.

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