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# RESOLUTION IN THIN-LAYER CHROMATOGRAPHY WITH SOLVENT OR ADSORBENT PROGRAMMING

## COMPARISONS WITH COLUMN CHROMATOGRAPHY AND NORMAL THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A general theory of sample resolution has been developed for various TLC techniques in which the composition of the developing solvent or adsorbent varies throughout separation. For the separation of complex samples containing many components of widely differing adsorptivities, it is predicted that gradient-layer thin-layer chromatography (TLC) should provide generally better separation than gradient elution TLC, and the latter technique should in turn be superior to polyzonal TLC. However, these generalizations must be qualified by certain practical considerations. The advantages of vapor-programmed TLC as recently described appear to be open to question. At the present time none of these TLC techniques can compete with gradient elution from columns as regards separation speed and resolution in a single separation.

## INTRODUCTION

In the separation of a sample by liquid–solid chromatography (LSC) one can choose from among a variety of different techniques: normal column chromatography or thin-layer chromatography (TLC), stepwise or gradient elution from columns or on plates (TLC), continuous or multiple development TLC, adsorbent gradients (TLC), and so forth. Apart from experimental convenience and the equipment available for separation, the main factors in the selection of a given procedure are separation speed and sample resolution. Until recently, however, comparisons of different LSC techniques with respect to relative speed and resolution have been difficult to make. Prior to 1967 little existed in the way of adequate theory or relevant experimental data. Recent experimental and theoretical studies[1–4] have clarified this situation with respect to normal column and TLC techniques, including continuous and multiple development TLC. It now appears that column separations in these cases have a significant advantage over TLC with respect to both separation speed and resolution, when each procedure has been experimentally optimized.

The difficulty in adequately separating many multicomponent samples by normal LSC (*i.e.* where the same solvent and adsorbent are used throughout separation) has given rise to a number of special techniques: stepwise or gradient elution from

columns, analogous solvent-programming techniques in TLC, gradient-layer TLC, polyzonal TLC, etc.*. Solvent programming in columns (gradient or stepwise elution) has been examined recently[6] with respect to separation speed and resolution, but comparable studies of related TLC procedures (see reviews of refs. 5 and 7) are so far lacking. In this paper we will develop a simple, idealized theory of resolution for these latter TLC procedures. Since separation time in TLC is normally fixed within narrow limits, we can ignore the possibility of simultaneously varying separation speed. In this attempt we recognize that the complexity of practical TLC systems (the latter techniques in particular)—and the resulting approximations which are required in any practical theory—will limit the validity of our final conclusions. On the other hand, this same experimental complexity simultaneously precludes the easy generalization of experimental TLC data and the direct comparison of different techniques. In the final analysis simple theory is necessary as an initial guide in attempting to understand these various chromatographic systems—particularly with regard to comparisons between different TLC and column procedures.

## GENERAL THEORY

Resolution in single-solvent separations with a fixed stationary phase (*i.e.* normal column or TLC procedures) is best defined by the relationship

$$R_s = \Delta d/2(\sigma_A + \sigma_B) \tag{1}$$

At the end of separation, $\Delta d$ is the spacing between the centers of two adjacent, adsorbed bands ($A$ and $B$), and $\sigma_A$ and $\sigma_B$ are their widths (standard deviations of the Gaussian curves). Eqn. (1) is directly applicable in TLC separations. For elution from a column, the quantities $\Delta d$, $\sigma_A$ and $\sigma_B$ are measured immediately before elution of the two bands from the column. For two narrow, closely adjacent bands—the case of greatest interest—it can be assumed that $\sigma_A = \sigma_B$, and $k_A \approx k_B$ ($k_A$ and $k_B$ are the partition ratios for bands $A$ and $B$; *i.e.* the ratio of total $A$ or $B$ in the stationary phase to total $A$ or $B$ in the mobile phase during separation). With these approximations it can be shown readily (*e.g.* ref. 2) that

$$R_s = (1/4)\ [(k_A/k_B)-1]\ \sqrt{N'}\ [k_B/(1+k_B)] \tag{1a}$$
$$\qquad\quad (a) \qquad\quad (b) \qquad\quad (c)$$

Here $N'$ is the number of theoretical plates in the bed length that have been traversed by $A$ or $B$ at the end of separation. For elution from a column, $N'$ is equal to the total number of plates ($N$) in the column. For separations by TLC, $N'$ is equal to $R_F \cdot N$, where $R_F$ refers to the average distance migrated by $A$ and $B$ relative to the solvent front, and $N$ is the number of plates in the adsorbent bed behind the solvent front. Resolution is seen in eqn. (1a) to be a product of three essentially independent factors: (a) a separation selectivity factor, (b) a bed efficiency factor, and (c) a function of the partition ratio of $B$ (or $A$, since $k_B \approx k_A$). The optimization of separation selectivity and efficiency in LSC has been discussed in detail[4,8]. For a given set of experimental conditions (*i.e.* single solvent and adsorbent), the optimum value of $k_B$ is 2 so that $R_F = 1/3$ and factor (c) is equal to $2/3$ (but see discussion of ref. 8).

When a given sample contains many components of widely differing migration

* For a description of these special TLC techniques see ref. 5 and following sections of this paper.

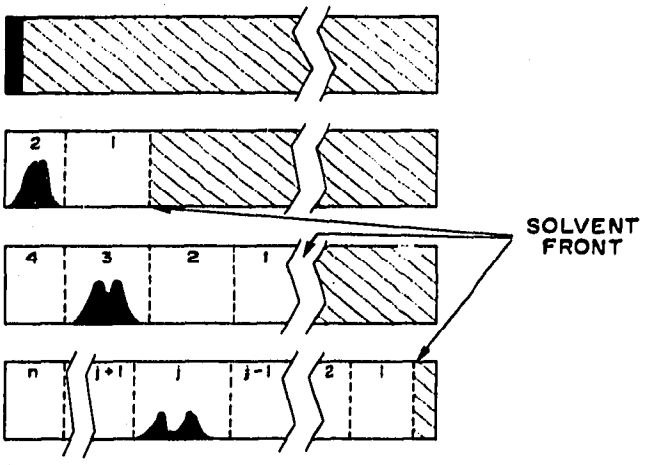
rates ($k$ values), eqn. (1a) predicts that weakly adsorbed components will be poorly resolved; *i.e.* factor (c) is small. Similarly, eqn. (1a) predicts that in TLC strongly adsorbed components will also be poorly resolved; *i.e.* $R_F$ and $N'$ are small. In elution from columns, strongly adsorbed bands are well resolved but require excessive separation times (this is equivalent to a loss in resolution per unit time). This general problem can be overcome by a systematic change in sample component $k$ values during separation, such that each component is separated under optimum conditions (*i.e.* $k \approx 2$). Sample $k$ values can be changed by variations in temperature (temperature programming), solvent (stepwise or gradient elution, polyzonal TLC, etc.) or adsorbent (gradient layer TLC). In column chromatography solvent programming is preferable to other techniques as a means of varying $k$ during separation[6]. For similar reasons it can be argued that temperature programming in TLC is not as effective as solvent programming*. The present theoretical treatment will therefore ignore the possibility of TLC temperature programming.

Experimental TLC separations are subject to a number of complications which would be quite difficult to include in a theoretical treatment of the present kind. These complications include the transfer of solvent between plate and vapor phase during separation, the development of solvent concentration gradients (*i.e.* varying ratios of solvent to adsorbent) across the plate in the direction of solvent flow, solvent demixing during separation, temperature effects (heat of wetting), and changes in adsorbent activity during separation (see ref. 4, sect. 13.2E). In the present examination, unless otherwise noted, each of these effects is ignored. This means that we assume all solvent transfer to the plate occurs by capillary flow up the plate, and the ratio of solvent to adsorbent at any point behind the solvent front is constant. These approximations have a significant effect on the validity of final quantitative data furnished by our theory, but any qualitative conclusions do not appear to be seriously compromised.

*Gradient elution thin-layer chromatography*

By gradient elution TLC we mean a separation in which the composition of solvent entering the bottom of the plate changes with time (as in gradient elution from columns). We can approximate any such continuous solvent program by a series of individual solvents 1, 2...$i$...$n$ of volumes $V_1$, $V_2$...$V_i$...$V_n$ and average partition ratios (for a given pair of bands $A$ and $B$) $k_1$, $k_2$...$k_i$...$k_n$. A given pair of sample bands $A$ and $B$ will be carried a certain distance along the adsorbent bed as a result of the passage of the first solvent volume ($V_1$) through the two band centers (see Fig. 1). Similarly the two bands will be carried still further along the adsorbent bed by passage of solvent 2 through the band centers. Finally, this process will end when the front of solvent 1 reaches the end of the adsorbent bed (or some arbitrary point short of the bed end). At this time the two bands will be surrounded by some intermediate solvent $j$. Assuming that $k_n$ is reasonably small, the two bands will have migrated a significant distance along the bed; *i.e.* neither $R_F$ nor $N'$ will be zero. If $k_1$ is reasonably large, and if $k$ decreases by regular steps in going from solvent 1 to $n$, migration of two bands will begin when $(k/1+k)$ decreases significantly below one and will accelerate as $k$ approaches zero (thus keeping the two bands well ahead of the very strong solvents in the latter part of the solvent program (for which $k$ equals zero). Consequently throughout separation the factor (c) of eqn. (1a) will be significantly

* This assumes no vaporization of mobile phase as temperature is increased. Flux gradient TLC represents an exception.



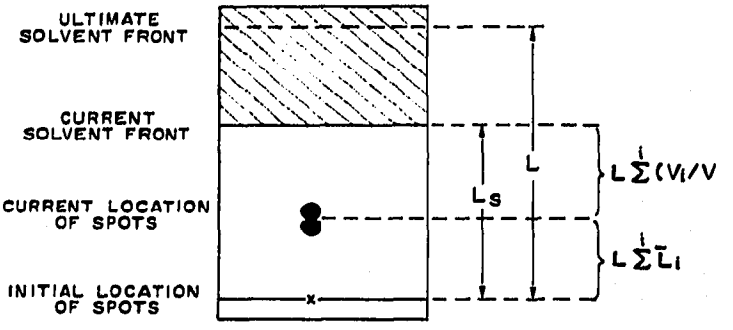




Fig. 1. Separation of a pair of sample components in gradient elution TLC.

Fig. 2. Gradient elution TLC after passage of the $i$-th solvent through the two bands of interest.

greater than zero. In this way significant sample resolution is maintained for both weakly and strongly adsorbing sample components, as long as factor (a) is not close to zero. It is only required that $k_1 > 0$ and $k_n/(1+k_n) < 1$.

The calculation of resolution in gradient elution TLC proceeds in essentially the same way as for solvent programming in column elution (see discussion of ref. 6). We begin by allowing the initial solvent volume $V_1$ to pass completely through the band centers of $A$ and $B$. As a result the two bands migrate some distance $L_1$ along the bed, given by

$$L_1 = L\ (V_1/V^0)/k_1 \tag{2}$$

$L$ is the total length of the bed (more accurately, the total length of the bed which lies between the initial point of sample application and the final solvent front), and $V^0$ is the volume of total solvent required to wet the bed length $L$. We next allow the second solvent volume $V_2$ to pass completely through the band centers of $A$ and $B$. As a result the two bands migrate an additional distance $L_2$ through the bed. This process is continued until the front of solvent 1 reaches the end of the bed or some predetermined point which marks the end of separation. For any solvent $i$, the fractional distance $L_i/L = \bar{L}_i$ migrated by $A$ and $B$ as a result of the passage of solvent $i$ through the two band centers is given as

$$\bar{L}_i = \frac{V_i/V^0}{k_i} \tag{2a}$$

The total distance migrated by the solvent front $L_s$, after passage of solvent $i$ through the two band centers, is given by eqn. (2b) (see Fig. 2).

$$L_s/L = \sum^{i} (V_i/V^0) + \sum^{i} \bar{L}_i \tag{2b}$$

At the completion of separation, $L_s/L$ equals one, so the condition that the two bands will be surrounded by solvent $j$ at the conclusion of separation (see Fig. 1) is

$$\sum^{j-1} [(V_i/V^0) + \bar{L}_i] < 1 < \sum^{j} [(V_i/V^0) + \bar{L}_i] \tag{2c}$$

The distance migrated by the two bands as a result of the passage of some fraction of

the $j$-th solvent through the band centers ($L_{j'}$) is calculated as follows. After the passage of solvents 1 through ($j-1$) across the band centers, the distance lying between the front of solvent 1 and the end of the bed (or final solvent front) $\Delta L$ is given by

$$\Delta L/L = 1 - \sum^{j-1} [(V_t/V^0) + \bar{L}_t] \tag{3}$$

The average $R_F$ value of the two bands in solvent $j$, $(R_F)_j$, is seen to be given by

$$\begin{aligned}(R_F)_j &= L_{j'}/\Delta L \\ &= 1/(1 + k_j)\end{aligned} \tag{3a}$$

The quantity $\bar{L}_{j'} = L_{j'}/L$ can be defined, and from the above relationship

$$\bar{L}_{j'} = (\Delta L/L)/(1 + k_j) \tag{4}$$

with $(\Delta L/L)$ calculable from eqn. (3). The average $R_F$ value of the two bands is then given as $(\sum^{j-1} \bar{L}_t) + \bar{L}_{j'}$. The resolution $R_s$ developed as a result of the migration of the two bands the total distance $\bar{L}_1 + \bar{L}_2 + \ldots \bar{L}_{j-1} + \bar{L}_{j'}$ can now be calculated in the same way that $R_s$ has previously been calculated for solvent programming in elution from columns (see ref. 6):

$$(R_s)^2 = \frac{N\,[(k_A/k_B) - 1]^2 \cdot Q^2}{16} \tag{5}$$

$N$ is the total number of theoretical plates in the bed length $L$, and the quantity $Q^2$ is given as

$$Q^2 = \frac{\left[\sum^{j} \left(\sum_{i \le m \le j} G_m \bar{L}_i k_i/(1 + k_i)\right)\right]^2}{\sum^{j} \left(\sum_{i \le m \le j} G_m^2\right) \bar{L}_i} \tag{5a}$$

The summation over $\bar{L}_i$ ends with the term $\bar{L}_{j'}$ (note the similarity of eqn. (5a) above and eqn. (6c) from ref. 6). The band compression factor $G_m$ is given as

$$G_m = k_{m+1}\,(1 + k_m)/k_m\,(1 + k_{m+1}) \tag{5b}$$

Eqn. (5a) permits us to calculate the number of effective theoretical plates $NQ^2$ in a separation by gradient elution TLC as a function of a particular solvent program (series of values of $V_t$ and $k_t$ for different sample bands) and value of $N$.

One of the more important characteristics of a solvent program in gradient elution TLC is its ability to provide comparable sample resolution (*i.e.* comparable values of $NQ^2$) for components of differing relative adsorption (differing values of $k_1$). In the case of gradient elution from columns, it has been shown[6] that so-called logarithmic solvent programs (eqn. 5c) give equal resolution for all sample components except those that are very weakly adsorbed (*i.e.* $k_1$ small):

$$\log k = \log k_1 - b(V/V^0) \tag{5c}$$

Here $V$ is the total volume of solvent which precedes solvent of partition ratio $k$, $k_1$ refers to the $k$ value for the first solvent in the program, and $b$ is seen to be a measure of gradient steepness; *i.e.* how fast $k$ changes with solvent volume $V$. We have calculated $Q^2$ of eqn. (5a) (by computer) as a function of $b$ for various logarithmic solvent programs in gradient elution TLC ($V_t$ small). The results of this calculation are shown in Fig. 3a as $Q^2$ *vs.* $R_F$ and in Fig. 4a as $Q^2$ *vs.* $\log k_1$. Assuming a typical

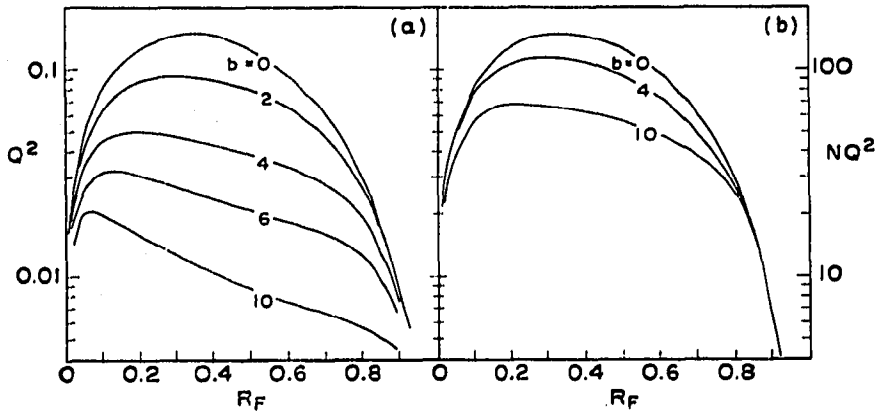



Fig. 3. Resolution in gradient elution TLC (a) and gradient-layer TLC (b) as a function of gradient steepness $b$ (logarithmic programs with $V_t$ small).

value of $N$ in TLC equal to 1000 (ref. 2) we have also indicated values of $NQ^2$ (so-called "effective theoretical plates") in Figs. 3 and 4.

What is the significance of these calculated values of $Q^2$ and $NQ^2$? First, the data of Figs. 3a and 4a show how the number of effective theoretical plates $NQ^2$ (and sample resolution) varies with the steepness of the solvent gradient $b$, differences in compound adsorptivity $k_1$ and the distance migrated along the plate ($R_F$). We see that average resolution along the plate (*i.e.* $NQ^2$) decreases with increasing steepness of the solvent gradient, just as in gradient elution from columns[6]. At the same time, however, a greater range of sample components (a greater range in $k_1$ values) can be separated with comparable resolution (Fig. 4a). Second, we see that a logarithmic solvent program does not provide equal values of $NQ^2$ for all sample components in TLC, in contrast with the case of gradient elution from columns[6]. To a certain extent this reflects "end effects" which are beyond our control in actual practice (*i.e.* the inevitable approach of $R_s$ to zero for $R_F$ values close to zero or one). A similar phenomenon was encountered in gradient elution from columns[6] for the case of weakly adsorbed sample components. For steep solvent gradients ($b > 4$) there is a systematic decrease in $NQ^2$ with increasing $R_F$ over most of the plate (Fig. 3a). This could be corrected (*i.e.* $NQ^2$ made more nearly constant) by changing the form of the solvent

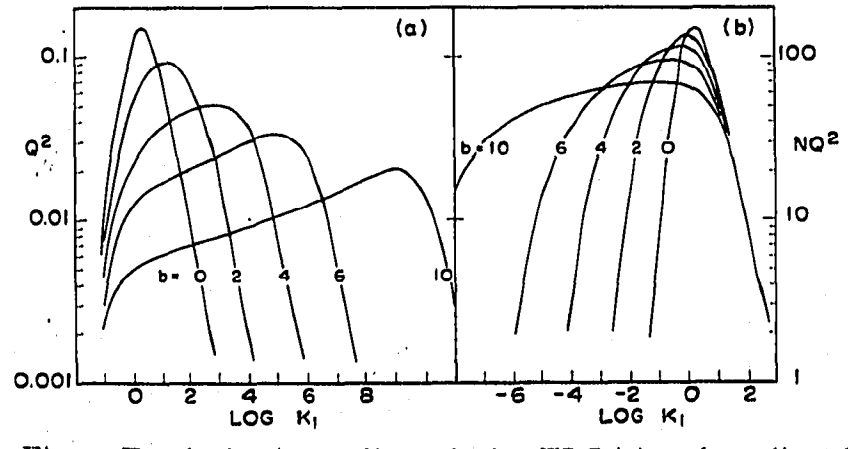



Fig. 4. Resolution in gradient elution TLC (a) and gradient-layer TLC (b) as a function of gradient steepness $b$ (logarithmic programs with $V_t$ small).

program so that $\log k$ changes more rapidly with $V$ in the latter part of the program (relative to a logarithmic solvent program). Finally, the values of $NQ^2$ which are provided by gradient elution TLC with steep solvent gradients ($b > 4$) are rather small (generally less than 30 effective plates). By contrast, gradient elution from columns easily provides in excess of 400 effective plates for all sample components, with comparable separation times[6]. Thus gradient elution TLC is very much less efficient than gradient elution from columns, when the latter is properly optimized, just as normal column elution is more efficient than normal TLC (*i.e.* fixed solvent throughout separation). Alternatively, comparable separations by gradient elution from columns can be obtained in much less time than by gradient elution TLC.

*Adsorbent gradients in thin-layer chromatography*

By an adsorbent gradient in TLC we mean a systematic change in the composition of the adsorbent (or adsorbent activity) along the plate. Adsorbent gradients can exist either parallel to the direction of solvent flow, or at right angles to solvent flow (see ref. 5). Both techniques have been referred to as "gradient-layer TLC". The use of adsorbent gradients at right angles to solvent flow is equivalent to using several different plates of varying adsorbent composition for an initial survey of optimum TLC separation conditions. This technique is outside the scope of the present treatment. The use of an adsorbent gradient parallel to solvent flow provides still another means of dealing with the problem of multicomponent sample separation in TLC. Thus if the initial part of the plate consists of very weak adsorbent, strongly adsorbing sample components will be separated on this section of the plate, and less strongly adsorbing components will pass through without separation. If the remainder of the plate is composed of adsorbent of continuously increasing activity (stronger retention of all sample components), the remaining sample components will be retained in various parts of the bed and separated there. In this way both strongly and weakly adsorbing sample components can be resolved in a single separation.

The calculation of resolution in gradient-layer TLC proceeds in much the same way as for gradient elution TLC. We begin with a bed divided into segments 1, $2 \ldots i \ldots n$ of relative lengths ($\bar{L}_i = L_i/L$) $\bar{L}_1, \bar{L}_2 \ldots \bar{L}_i \ldots \bar{L}_n$, and corresponding $k$ values (for a particular pair of adjacent bands $A$ and $B$) $k_1, k_2 \ldots k_i \ldots k_n$. Passage of some volume $V_1$ of solvent (same solvent throughout) will suffice to carry the two bands to the end of bed segment 1, with

$$V_1/V^0 = \bar{L}_1 k_1 \tag{6}$$

Similarly, passage of some volume $V_2$ of solvent suffices to carry the two bands to the end of bed segment 2. The total distance migrated by the solvent front $L_s$, after passage of the two bands through the first $i$ bed segments is given by eqn. (2b). The condition that the two bands will lie in bed segment $j$ at the end of separation is given by eqn. (2c). The distance migrated by the two bands in the $j$-th bed segment is given by eqn. (4), and the average $R_F$ value at the end of separation is equal to $({}^{j}\bar{\Sigma}^{1}\ \bar{L}_i) + \bar{L}_{j'} = \bar{L}$ (as for gradient elution TLC). The resolution $R_s$ developed as a result of the migration of the two bands the total distance $({}^{j}\bar{\Sigma}^{1}\ \bar{L}_i) + \bar{L}_{j'}$ can now be calculated in essentially the same way that $R_s$ was calculated in gradient elution from columns or in gradient elution TLC. The resulting expression is the same as that given in eqns. (5) and (5a), except that the band compression factor $G_m$ is given by

$$G_m = (1 + k_m)/(1 + k_{m+1}) \tag{7}$$

The latter expression can be derived in the same way as eqn. (5b) was derived originally in ref. 6. These relationships allow us to calculate the effective theoretical plates $NQ^2$ in gradient-layer TLC as a function of sample adsorptivity $k_1$ and the adsorbent activity program ($k$ as a function of position on the plate). In Figs. 3b and 4b we have plotted the results of such a calculation for a logarithmic adsorbent activity program (*i.e.* $\log k = \log k_1 + b[x/L]$, where $x$ is the distance along the plate from the point of sample application). The data of Figs. 3b and 4b show a general resemblance to the calculations for gradient elution TLC: steeper adsorbent activity gradients (larger values of $b$) provide less resolution of adjacent bands, but a wider range of sample components can be separated; the logarithmic adsorbent activity program does not provide exactly equal resolution at all positions on the plate; sample resolution is significantly poorer than comparable separations by gradient elution from columns. The major difference between gradient elution and gradient-layer TLC is generally better resolution by the latter technique. For large values of $b$ and conditions that provide significant resolution for a comparable range in $k_1$ values, gradient-layer TLC can offer from two to three times more effective plates than in the case of gradient elution. Thus our simple theory predicts that gradient-layer TLC is superior to gradient elution TLC.

Although our simple theory indicates a clear-cut superiority of gradient layer TLC over gradient elution TLC, in actual practice this will not always be the case. The problem is an experimental one, namely the great difficulty in preparing adsorbent gradients with $b > 4$ (see below). When $b < 4$, the relative advantage of gradient layer TLC with respect to resolution is considerably reduced (*i.e.* $NQ^2$ for gradient layer TLC approaches that for gradient elution TLC at similar values of $b$). Furthermore, the limitation $b < 4$ reduces the range in sample components (*i.e.* $k_1$ values) which can be separated on a single plate, which is a further limitation on gradient layer TLC.

We should note in passing that adsorbent gradients can be achieved in several different ways. An active adsorbent (*e.g.* silica gel) can be mixed with varying proportions of an inactive solid (*e.g.* Kieselguhr). This is a simple procedure, but the maximum range in $k$ values (proportional to concentration of strong adsorbent) is limited to about $10^2$. Greater dilution of the strong adsorbent would result in too low a capacity and overloading of the initial part of the plate. The adsorbent bed can also be exposed to solvent vapors in special devices[9,10] which permit different vapors to contact different parts of the plate, creating an adsorbent activity gradient across the plate. Presumably a similar device, loaded with adsorbent of varying water content, would also permit varying deactivation of the plate with water vapor (*e.g.* ref. 11).

*Polyzonal thin-layer chromatography*

In this technique the advantages of gradient elution TLC can be obtained by using a multicomponent solvent mixture of a type which will undergo solvent demixing during separation (see discussion of ref. 5). In the simplest case, that of a two component solvent system $\underline{a}$–$\underline{b}$ ($\underline{b}$ the stronger adsorbing solvent), selective adsorption of $\underline{b}$ occurs during the advance of solvent $\underline{a}$–$\underline{b}$ up the plate. When the difference in solvent strengths of $\underline{a}$ and $\underline{b}$ (*i.e.* their relative adsorptivities or $\varepsilon^0$ values; see ref. 4) is sufficiently great, a secondary solvent front will be observable between the main

solvent front and the point of sample application. These two solvent fronts are termed the $\alpha$ front (primary front) and $\beta$ front (secondary front), respectively. The composition of solvent lying between the $\alpha$ and $\beta$ fronts ($\alpha$ zone) will be pure $\underline{a}$, while the composition of solvent between the $\beta$ front and the point of sample application ($\beta$ zone) will be the original solvent $\underline{a}$–$\underline{b}$. The $k$ value of a given compound will be greater in the $\alpha$ zone than in the $\beta$ zone. As a result, strongly adsorbing sample components will tend to migrate in the $\beta$ zone, with significant resolution, while weakly adsorbing components will migrate in the $\alpha$ zone (again with significant resolution). Thus polyzonal TLC appears to offer the same advantages as gradient elution TLC. As many as three separate solvent fronts ($\alpha$, $\beta$, $\delta$) have been achieved in polyzonal TLC[5].

To appreciate the differences between polyzonal TLC and gradient elution TLC as regards sample resolution, we will compare resolution in each case for the simplest possible analogous systems: a two component solvent mixture $\underline{a}$–$\underline{c}$ in polyzonal TLC *vs.* gradient elution first with $\underline{a}$ then with $\underline{c}$ ($\underline{c}$ chromatographically equivalent to $\underline{a}$–$\underline{b}$, except no solvent demixing). We will make the following assumptions:

(1) equal lengths for the final $\alpha$ and $\beta$ zones in the polyzonal TLC case;

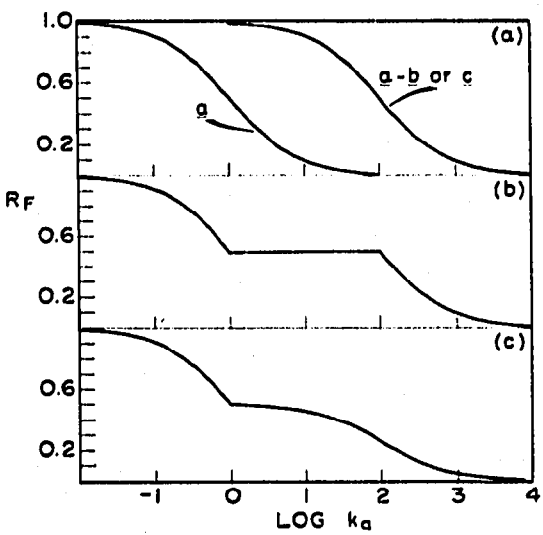
(2) equal volumes of $\underline{a}$ and $\underline{c}$ pass through the point of sample application in the gradient elution case;

(3) the solvent level in contact with the bottom of the plate coincides with the point of sample application (but note the additional possibilities discussed in ref. 5);

(4) solvent demixing is quite pronounced, so that the $\beta$ front sharply divides solvent of original composition $\underline{a}$–$\underline{b}$ from pure $\underline{a}$ (the $\alpha$ zone); sample $k$ values will therefore change abruptly across the $\beta$ front by some large factor.

In our model calculation we will further assume an arbitrary (large) ratio of $k$ values in the two zones ($\alpha$ and $\beta$): $k_\alpha/k_\beta = 100$. Similarly we will assume for solvents $\underline{a}$ and $\underline{c}$ that $k_{\underline{a}}/k_{\underline{c}} = 100$. First, consider sample $R_F$ values in pure $\underline{a}$ or pure $\underline{c}$ ($\equiv \underline{a}$–$\underline{b}$). These are plotted in Fig. 5a *vs.* values of $k_{\underline{a}} = k_\alpha$. For the same compound (*i.e.* a given value of $k_{\underline{a}}$), $R_F$ values are of course smaller in solvent $\underline{a}$ than in solvents $\underline{c}$ or $\underline{a}$–$\underline{b}$. Next consider $R_F$ values as a function of $k_{\underline{a}}$ in our two-solvent polyzonal TLC system. For compounds with $k_{\underline{a}}$ values less than one, the $R_F$ value of the compound in pure $\underline{a}$ is greater than 0.5, and the compound will always migrate in the $\alpha$ zone. Consequently for $k_{\underline{a}} \leqslant 1$, $R_F$ values in our model polyzonal TLC system will be equal to $1/(1+k_{\underline{a}})$; *i.e.* identical to $R_F$ values which would result if pure $\underline{a}$ was the developing solvent throughout separation. Similarly compounds with $k_{\underline{a}}$ values greater than 100 ($R_F$ in solvent $\underline{a}$–$\underline{b} \leqslant 0.5$) will always migrate in the $\beta$ zone, and their $R_F$ values will be equal to $1/(1 + 0.01\ k_{\underline{a}})$; *i.e.* the same as would result for solvent $\underline{a}$–$\underline{b}$ (or $\underline{c}$) in the absence of solvent demixing. Sample components with $k_{\underline{a}}$ values between 1 and 100 will migrate unresolved at the $\beta$ front. $R_F$ *vs.* $k_{\underline{a}}$ is plotted in Fig. 5b for our model polyzonal TLC system.

In the case of gradient elution TLC with solvent $\underline{a}$ followed by solvent $\underline{c}$, compounds with $k_{\underline{a}}$ values less than one always migrate in the $\underline{a}$ solvent zone, and their $R_F$ values are the same as in elution with pure $\underline{a}$ throughout separation. Compounds with $k_{\underline{a}} > 1$ migrate a certain distance across the plate as a result of the passage of solvent $\underline{a}$ across the band center (Eqn. 2). Then these bands are overtaken by solvent $\underline{c}$, compressed by the factor $G_m$ (Eqn. 5b), and migrate a certain distance as a result of passage of $\underline{c}$ through the band center. The total distance migrated by a band with $k > 1$ can be calculated as in the preceding section on gradient elution TLC



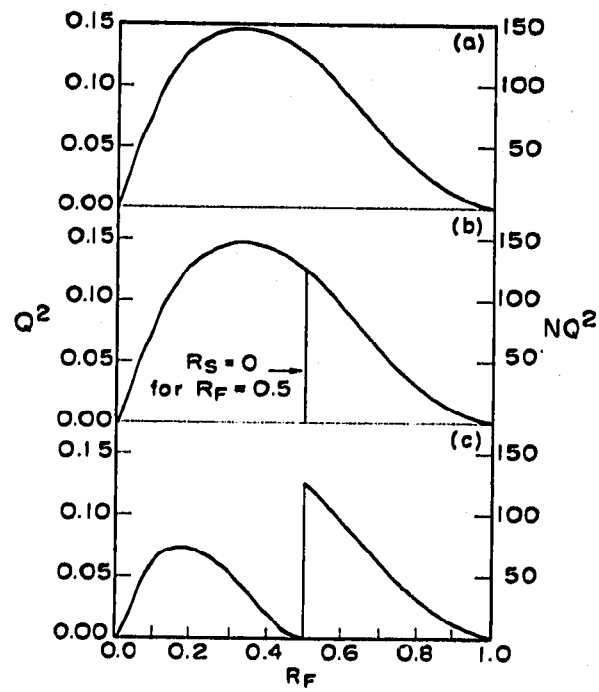




Fig. 5. $R_F$ values *versus* $k_a$ in normal TLC (a), polyzonal TLC (b) and gradient elution TLC (c); model system with two solvents ($k_a/k_b = 100$).

Fig. 6. Resolution in normal TLC (a), polyzonal TLC (b) and gradient elution TLC (c); same systems as in Fig. 5.

(Eqns. 2a–4). The resulting $R_F$ *vs.* $k_a$ plot is shown in Fig. 5c.

Now consider the evaluation of resolution in these two cases; *i.e.* $NQ^2$ *vs.* $k_a$ in polyzonal and gradient elution TLC. First, we must calculate $NQ^2$ in single-solvent TLC as a function of $k$: $NQ^2 = NR_F\, k/(1 + k)^2$ (Eqn. 1a). This function is plotted *vs.* $R_F$ in Fig. 6a and *vs.* $k_a$ in Fig. 7a. Now in polyzonal TLC, the resolution of compounds with $k_a$ less than one is the same as for single-solvent elution with *a* throughout. Similarly, the resolution of compounds with $k_a$ greater than 100 is the same in polyzonal TLC as for single-solvent elution with *a*–*b* throughout, assuming no solvent demixing (see above discussion of $R_F$ values in polyzonal TLC). For compounds with $1 \leqslant k_a \leqslant 100$, $NQ^2$ is equal to zero since these compounds are bunched together with $R_F$ equal 0.5. A plot of $NQ^2$ *vs.* $R_F$ is thus essentially the same for our model polyzonal TLC case (Fig. 6b) as for normal TLC elution (Fig. 6a). The corresponding plot of $NQ^2$ *vs.* $k_a$ (polyzonal TLC) is shown in Fig. 7b.

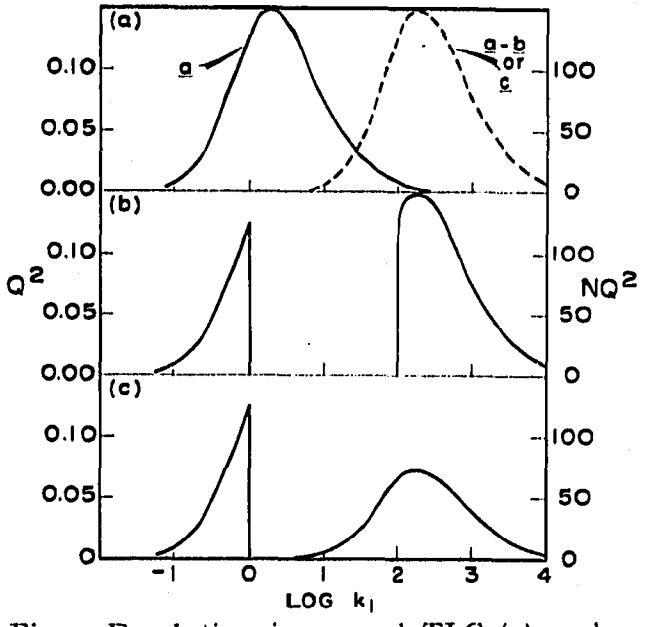
In the case of gradient elution with *a* followed by *c*, compounds with $k_a \leqslant 1$ again have the same resolution as for development with solvent *a* throughout. For compounds with $k_a > 1$, resolution can be calculated as previously described (Eqn. 5). Plots of $NQ^2$ *vs.* $R_F$ and $NQ^2$ *vs.* $k_a$ are shown in Figs. 6c and 7c, respectively.

A simple comparison of $NQ^2$ *vs.* $R_F$ in Fig. 6 suggests that equivalent resolution is provided by normal (single-solvent) and polyzonal TLC, with somewhat inferior resolution for gradient elution TLC. This is an oversimplication, however, as shown in Fig. 7. Here we see that gradient elution TLC provides adequate resolution for the same range of $k_a$ values (*i.e.* sample components) as provided by polyzonal TLC, plus significant resolution for components with $10 \leqslant k_a \leqslant 100$, where polyzonal TLC provides no resolution whatsoever. Thus in comparing the two techniques, we see

that gradient elution TLC provides significant resolution for a greater range of sample components, at the price of somewhat lower resolution than polyzonal TLC for some of these components. Judged from the standpoint of comparable resolution for all sample components (which is one of the main objectives of special techniques for multicomponent samples), gradient elution TLC is superior to polyzonal TLC*. We also see in Fig. 7a that two normal TLC separations (with solvents *a* and *c*) provides better overall sample resolution than either of the two other techniques.

*Vapor-programmed thin-layer chromatography*

This is a recently introduced technique[9,10] for carrying out TLC separations on a plate which has been initially exposed to the vapors of a series of different solvents. Because of varying deactivation by adsorbed solvent, the activity of the plate varies from one end to the other. Exposure of a plate section to a strong solvent such as methanol leads to highly deactivated adsorbent and small $k$ values, and vice versa for weak solvents such as the hydrocarbons. In principle vapor-programmed TLC could be used in the same way as gradient-layer TLC, and the resulting theory of resolution would be the same for these two techniques (Figs. 3b and 4b would describe vapor-programmed TLC). Actually vapor-programmed TLC has been suggested[9] for the separation of difficultly separable mixtures (similar $k$ values), rather than multicomponent mixtures of widely different $k$ values. In pursuit of this objective, it has been suggested that the adsorbent activity should decrease in the direction of solvent flow (negative value of $b$), rather than increase as in normal gradient-layer TLC.



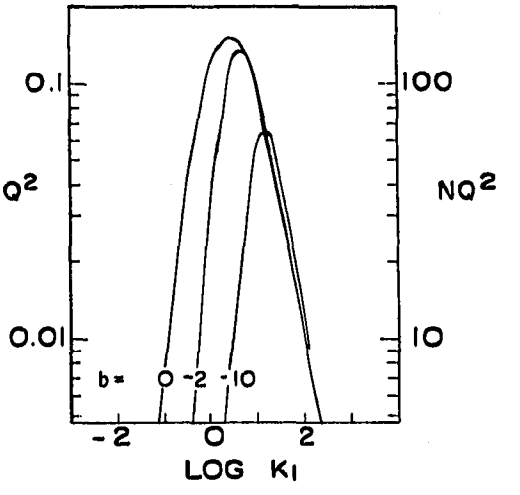




Fig. 7. Resolution in normal TLC (a), polyzonal TLC (b) and gradient elution TLC (c); same systems as in Fig. 5.

Fig. 8. Resolution in gradient-layer TLC with negative adsorbent gradients.

* However for multicomponent solvent systems where several fronts are formed and the individual fronts are not sharply defined (*i.e.* there is a continuous transition from one zone to the next over a finite part of the TLC plate), it can be shown that polyzonal TLC gradually merges into gradient elution TLC so far as relative resolution is concerned. That is, in this case gradient elution and polyzonal TLC provide comparable resolution for similar values of $b$.

Fig. 8 shows the calculated resolution for such negative activity gradients. We see that average resolution ($NQ^2$) decreases for increasingly negative $b$ values, and the range of sample components (*i.e.* range of $k$ values) that can be efficiently separated (large $NQ^2$) also decreases as $b$ becomes more negative. Thus a negative activity gradient of this type actually works in the opposite direction to that desired. A negative activity gradient does provide greater displacement of peak centers for bands near the center of the plate, because bands tend to migrate more rapidly (relative to the solvent front), the further they progress along the plate. This gives the appearance of improved sample resolution in some cases, particularly when the bands are well resolved in the absence of an adsorbent activity gradient. As shown in Fig. 8, however, real resolution (as measured by the ability to separate closely adjacent bands in high purity) becomes poorer for negative activity gradients.

In addition to a negative adsorbent activity gradient for vapor-programmed TLC, an alternation of active and inactive adsorbent sections along the plate has also been suggested[9]. This is quite similar to the negative solvent gradient or "polarity reversal" that has been used (*e.g.* ref. 12) to provide greater displacement of closely adjacent elution peaks. The principle of the latter technique is as follows. As soon as the first of two bands leaves the column, a much weaker solvent overtakes the second band, freezing it at the end of the column. Eventually a stronger solvent is used to elute the second band, and the two bands then appear as widely separated peaks in the elution chromatogram. It must be emphasized strongly that the latter technique—and the alternation of adsorbent activity in vapor-programmed TLC—does not provide any real gain in resolution. The relative contamination of each band by the other is the same despite their differing positions in the chromatogram. The only possible advantage of this artificially enhanced peak separation is that the two bands may be more easily recovered at the end of separation, with less chance of further intermixing as a result of manipulation during recovery.

In summary, vapor-programmed TLC could be a useful alternative to gradient-layer TLC, if the solvent vapors provide decreased adsorbent activity in the direction of development. The use of negative adsorbent activity gradients and the alternation of adsorbent activity appears to work at cross purposes to improved sample resolution in the general case.

## DISCUSSION

The present theoretical treatment suggests that the four TLC techniques we have examined can be arranged in an order of decreasing general performance: gradient-layer TLC (best), gradient elution TLC, polyzonal TLC and vapor-programmed TLC (worst). With suitable modification, however, vapor-programmed TLC should provide separations comparable to those by normal gradient-layer TLC. On the other hand, practical considerations make it difficult to achieve the full potential of gradient layer TLC, so that in many cases gradient elution TLC will be the preferred technique. None of these TLC techniques can compete with stepwise or gradient elution from columns in terms of speed or sample resolution, when the column procedures have been fully optimized[6,8]. Likewise none of these TLC procedures ever exceeds normal (single-solvent) TLC with respect to maximum resolution: *i.e.* $Q^2 = 0.15$ for $k$ equal 2. As the solvent or adsorbent gradients become less steep ($b \rightarrow 0$),

the resolution of these various procedures approaches that of normal TLC. As a result the separation of multicomponent samples (large range in $k$ values) with maximum resolution (large $NQ^2$) is best carried out by compositing the results of several different normal TLC separations (*e.g.* Fig. 7a—with solvents *a* and *c*—*vs.* Fig. 7b or 7c). Each of these individual TLC separations can be varied to provide optimum values of $k$ (equal 2) for the different pairs of closely adjacent bands in the sample, so that $NQ^2$ for the overall separation is equal to about 150 effective plates. Sample resolution could be further improved by as much as a factor of 6 (to about 1000 effective plates $NQ^2$) by carrying out the individual separations with continuous development[4], but this would involve a prohibitive amount of work for a given sample when several such separations are required.

It should be emphasized that we have focused attention on effective plates ($NQ^2$) and ignored separation selectivity $[(k_A/k_B) - 1]$; see eqn. (1a). While there is no reason to expect that separation selectivity will be consistently better in any one of these special TLC systems (*i.e.* gradient-layer *vs.* gradient development, etc.), separation selectivity will in general not be the same. Thus it is quite possible to observe better sample resolution with a technique that provides a smaller value of $NQ^2$, as a result of larger differences in $[(k_A/k_B) - 1]$. In general, however, we should expect better sample resolution in those techniques where $NQ^2$ is predicted to be larger. The choice of gradient-layer over gradient development TLC for a given separation is therefore likely to be correct, but will not always be so.

Another point which should be stressed is that many complex samples do not require a high separation efficiency ($NQ^2$ value) for their satisfactory separation, because $k_A/k_B$ is relatively large for all pairs of adjacent bands. Even relatively inefficient procedures (*e.g.* polyzonal TLC) can provide adequate separations in such cases. Therefore the ability of a given technique to provide reasonable separation of a particular multicomponent mixture is not an accurate criterion of the overall utility of that procedure for more difficult separations.

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