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NEW LOOK AT SOLVENT STRENGTH, SELECTIVITY, AND CONTINUOUS DEVELOPMENT

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CONTENTS

1. INTRODUCTION

Choice of solvent is the primary task of the thin-layer chromatographer[1]. The solvent should be adequately selective. The strength of the solvent is also important. However, no clear consensus has been available to the practical thin-layer chromatographer concerning choice of solvent strength.

In particular, the directives that can be gleaned from the literature regarding decrease of solvent strength as a practical technique for increasing selectivity are

variously vague, or obscure, or contradictory, or premature. We consider these deficiencies in turn.

Touchstone and Bobbins[2] state that "a decrease in (solvent strength) will increase separation and decrease R_F". However, this statement neither indicates quantitatively what really can be expected nor suggests any practice other than normal for accomplishing the effect. (Under Results and Discussion, we develop and evaluate quantitative expressions for the effect and suggest a new way to make it practically useful.) Such directives need not be vague: research data on the effects of solvent strength decrease are well established and plentiful. However, they have for all practical purposes been effectively hidden.

In order to test a molecular model of adsorption chromatography, Soczewinski and co-workers have since 1969 been studying many systems of compounds[3–9]. For this they have used solvent strength decrease. However, their results have all been expressed in R_M. Now, R_M is the logarithm of $[(1-R_F)/R_F]$[10,11]. Logarithmically related to the inverse of R_F, not easily visualized, the R_M expression was essentially deleted from the second edition of Stahl's book[12] and is not even mentioned in the text of Touchstone and Bobbins[2]. Obviously, R_M is not a working tool of the practical thin-layer chromatographer, who is also not much interested in adsorption models. In consequence, the highly pertinent conclusions of Soczewinski and co-workers on the solvent strength–selectivity relationship have been neither heard nor applied by most of those who might use them in practice. These conclusions, stated presently, contradict Snyder's, which in contrast were explicit and expressed in R_F.

Saunders and Snyder[13] wrote, "In normal TLC it can be shown that an optimum value of R_F is equal to 1/3". Snyder had earlier elaborated on this in his "Principles of Adsorption Chromatography"[14]. For example: "(Although) a change in solvent strength offers a means of separating overlapping sample bands, ...this technique is somewhat limited in its practical usefulness (because) optimum separation generally occurs for a narrow range of K^0 values ... corresponding to a solvent of given strength... (For) thin-layer chromatography, this is a particularly severe limitation"[15].

These statements express the conclusion from a derivation that was aimed at finding the distribution coefficient —and thus the R_F— that maximizes resolution. For the derivation, two assumptions were made. The crucial one hinged on selectivity, which Snyder had just defined: "the ratio K_1/K_2, which we will refer to as the selectivity of the adsorption system, *i.e.*, the ability of the system to separate the centers of bands 1 and 2"[16]. The assumption was then stated, as follows: "...we assume that selectivity ... remain(s) unchanged with varying sample K values"[17]. We shall show that that assumption does not follow from theory and is contrary to evidence; the assumption is wrong. So, therefore, is the unduly restrictive conclusion. We return to the work of Soczewinski and co-workers.

As mentioned, Soczewinski and co-workers have studied many compounds that comprise a number of chemically diverse systems. They have expressed their studies in plots of R_M *versus* log X_S, where X_S is the mole fraction of one or another polar solvent in a binary mixture. The other component of the binaries is always the non-polar cyclohexane. They commented[9] in 1975, "Most R_M *vs.* log X_S plots spread fanwise with the dilution of the polar solvent, a tendency observed already in earlier papers (refs. 4, 6 and 8 cited here); the selectivity of separation is thus generally

higher for lower values of X_S. There are also exceptions from this rule; for example, for isomeric dinitrobenzenes the plots spread in the opposite direction and better selectivity is obtained for more polar solvent mixtures". Thus, although there are exceptions, Soczewinski and co-workers have found and concluded from wide experience that selectivity generally increases with solvent strength decrease. (Ironically, as we have indicated and shall see, the extensive experimental and theoretical work of Snyder leads to the same conclusion.)

The prime difficulty in exploiting this method of increasing selectivity has been taken to be the fact that as solvent strength decreases, R_F values in response decrease in an essentially exponential fashion. Because of this, Thoma[18] abandoned the approach as theoretically attractive but experimentally impractical. The difficulty can actually be readily overcome in TLC by continuous development through a short bed. As will be seen, separations then need take no longer than usual, and there is in addition a bonus: spot detectability increases by perhaps an order of magnitude.

2. EXPERIMENTAL

A. TLC plates

Camag (New Berlin, Wisc., U.S.A.) silica gel G plates were used. For conventional developments they were cut into pieces 10 × 20 cm and for the spotting study and the continuous developments into squares 10 × 10 cm and rectangles 2.5 × 10 cm.

B. Solvents

Tuned Solvents (Regis Chemical Co., Morton Grove, Ill., U.S.A.) were used for solvent strengths 0.20, 0.25, 0.30 and 0.35. These mixtures consisted of relevant proportions of chloroform and carbon tetrachloride. Pure chloroform, without preservative, was used for solvent strength 0.40; pure carbon tetrachloride was used for solvent strength 0.18. A mixture of pure hexanes was used to make the 1:1, 2:1, 3:1 and 4:1 dilutions of chloroform.

C. Pipettes

Camag calibrated 1–5-μl pipettes were used for spotting.

D. Development chambers

Conventional developments were carried out in a conventional glass tank with a ground-glass lid.

Continuous developments were carried out in a glass tank approximately 7.5 cm deep inside. The tank lid was equipped with sliding cushioned jaws over a slot in the lid, to allow the plates to protrude without permitting vapor leakage from within the tank. "SB/CD" chambers, similar in function but considerably simpler in design and more versatile, are now available from Regis Chemical Co.

E. Dyes and dye solutions

The dyes used were Oil Orange, Butter Yellow, Sudan Green and Sudan III. For the plates shown in Fig. 1, the dyes were spotted from benzene solutions

just concentrated enough to allow the developed spots to be seen by eye. For the plates shown in Fig. 2, the dyes were spotted from carbon tetrachloride solution that contained 0.1 % (w/v) of each dye.

For the plates shown in Figs. 3 and 4, only Oil Orange and Sudan Green were used. From these, a solution in benzene was prepared to yield approximately equal photographic sensitivity to each of the two spots developed from the mixture (this required a high Sudan Green to Oil Orange ratio). From this benzene solution, exactly 2 ml were pipetted into each of two vials and taken to dryness under a gentle stream of nitrogen applied repeatedly until dryness seemed assured. Exactly 4 ml of chloroform were then pipetted into one of these vials, and exactly 4 ml of carbon tetrachloride into the other. Each vial thus contained identical weight-per-volume concentrations of the two dyes. We call these *unit* concentrations. From the chloroform and carbon tetrachloride solutions of unit concentration, corresponding solutions of exactly 1/5 and 1/25 concentrations were prepared, giving six solutions in all.

F. Spotting, development and photographic comparability

For Fig. 1, all spots were made from 1-μl depositions of relevant dye solutions. The spots for the dye mixtures were made by overspotting, with intermediate drying. All of the 10 × 10 cm plates shown in Fig. 1 were developed by continuous development for the times and with the solvents of solvent strength marked in the upper left-hand corner of each plate. The line of emergence of each plate from within the chamber was approximately 7.3 cm from the origin and 7.8 cm from the solvent level at the start of the development.

For Fig. 2, all spots were made from 1-μl depositions of the carbon tetrachloride solution that contained 0.1 % (w/v) of each dye. For each developing solvent, two plates were prepared and developed simultaneously. One plate was removed when the slowest spot (Sudan III) had moved 5 mm from the origin. The other plate was allowed to develop further until the whole four-spot chromatogram was approximately centered on the 7–8-cm chromatographic bed.

For Fig. 3, two plates were spotted, each with 1-μl volumes of the unit, 1/5 and 1/25 concentrations of the chloroform and carbon tetrachloride solutions, giving six spots per plate. The 10 × 20 cm plate was then developed conventionally for 90 min with chloroform as developing solvent. The 10 × 10 cm plate was developed for 35 min by continuous development on to a line approximately 2.5 cm from the solvent level and 1.1 cm from the origin. For this continuous development, carbon tetrachloride was used.

For Fig. 4, a grid of spots was prepared. As marked, rows corresponded to spot volumes of 1, 2 and 5 μl. Also as marked, columns corresponded to solvent (three columns each for the chloroform and carbon tetrachloride solutions) and solution relative concentrations (unit, 1/5 and 1/25).

The photographic sensitivity for the photographs depicted in Fig. 3 and shown in Fig. 4 was held as constant as possible. The plates were photographed immediately after either development and drying (Fig. 3) or spotting (Fig. 4).

For the determination of R_F values, conventional developments were made on the 10 × 20 cm plates, usually for a solvent advance of about 18 cm. When developments were expected to take more than 1 h, the lid was sealed on with tape to prevent

vapor leakage. From these determinations, the following regression lines were calculated, where $R_M = \ln[(1-R_F)/R_F]$;

Oil Orange: $R_M = -12.7\,\varepsilon + 5.75$;
Butter Yellow: $R_M = -18.2\,\varepsilon + 7.98$;
Sudan Green: $R_M = -21.1\,\varepsilon + 8.97$;
Sudan III: $R_M = -18.8\,\varepsilon + 9.24$.

In Table 1 are presented the R_F values that were measured and the corresponding R_F values that were calculated from these equations.

TABLE 1

OBSERVED AND CALCULATED R_F VALUES

Solvent strength ε	*Oil Orange*		*Sudan Green*		*Sudan III*		*Butter Yellow*	
	Observed	*Calc.**	*Observed*	*Calc.**	*Observed*	*Calc.**	*Observed*	*Calc.**
0.40	0.372	0.348	0.369	0.374	0.161	0.153	0.331	0.388
0.35	0.187	0.214	0.158	0.172	0.0601	0.0657	0.149	0.199
0.30	0.120	0.126	0.0655	0.0674	0.0270	0.0267	0.0687	0.0882
0.25	0.0735	0.0710	0.0350	0.0245	0.00970	0.0106	0.0413	0.0364
0.20	0.0449	0.0390	0.00898	0.00867	0.00599	0.00417	0.0180	0.0145
0.18	0.0279	0.0300	0.00447	0.00570	0.00223	0.00287	0.00559	0.0100

* Calculated from regression line derived from solvent strength–observed R_M pairs.

3. RESULTS AND DISCUSSION

A. Selectivity versus solvent composition and strength

(a) Expressions

Soczewinski and co-workers. The data of Soczewinski and co-workers are presented as plots of R_M *versus* $\log X_S$. The plots are usually straight lines. Actually of negative slope, the plots are by their convention presented with positive slope[7].

The straight-line plots of negative slope suggest that

$$R_M = \text{constant} - m \log X_S$$

Thus, as the molar fraction (X_S) of the stronger solvent in a binary mixture with a completely non-polar solvent approaches zero, the second term should approach unity and the R_M value, the constant *minus* m. If the slope, m, differs from one solute, S, to another, a fan-shaped set of plots should appear, opening to the lower right.

So we have that such a plot is in fact usually a straight line of negative slope m:

$$R_M = -m \log X_S + C \tag{1}$$

For two solutes, A and B, we have

$$R_{M,A} = -m_A \log X_S + C_A \tag{2}$$

and

$$R_{M,B} = -m_B \log X_S + C_B \tag{3}$$

As Soczewinski *et al.*[9] commented, "Most R_M *vs.* log X_S plots spread fanwise with the dilution of the polar solvent...". Such a negatively sloped fanwise spread for the plots of solutes A and B would have $R_{M,A} > R_{M,B}$ and $(-m_B) < (-m_A)$, *i.e.*, $|m_B| > |m_A|$.

To represent the dilution they speak of, let us introduce binary mixtures 1 and 2. We will have binary mixture 1 be the more dilute solvent; thus $X_{S,2} > X_{S,1}$.

With this in mind, we subtract eqn. 3 from eqn. 2 and express the difference once for mole fraction $X_{S,1}$ and once for mole fraction $X_{S,2}$:

$$(R_{M,A} - R_{M,B})_1 = -|\Delta m| \log X_{S,1} + \Delta C \tag{4}$$

$$(R_{M,A} - R_{M,B})_2 = -|\Delta m| \log X_{S,2} + \Delta C \tag{5}$$

In these equations, $\Delta m = (-m_A) - (-m_B) = m_B - m_A = -|\Delta m|$. We shall wish to have the minus sign expressly stated and the value of Δm understood as positive. Therefore, to avoid ambiguity, we have expressed Δm as $(-|\Delta m|)$ in eqns. 4 and 5, and also henceforth shall use $|\Delta m|$.

We now subtract eqn. 5 from eqn. 4 and rearrange:

$$(R_{M,A} - R_{M,B})_1 = (R_{M,A} - R_{M,B})_2 + \log \left(\frac{X_{S,2}}{X_{S,1}}\right)^{|\Delta m|} \tag{6}$$

Whereas $R_M = \log [(1 - R_F)/R_F]$, for those who think in terms of R_F eqn. 6 can be more usefully expressed:

$$\left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1 - R_{F,A}}{1 - R_{F,B}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1 - R_{F,A}}{1 - R_{F,B}}\right)_2 \left(\frac{X_{S,2}}{X_{S,1}}\right)^{|\Delta m|} \tag{7}$$

[Note again the inverse relationship of R_M and R_F; if $R_{M,A} > R_{M,B}$, $(R_{F,B}/R_{F,A}) > 1$.]

We shall examine presently whether either or both of the $(1 - R_{F,A})/(1 - R_{F,B})$ multipliers can for most purposes be dispensed with. The expression without them has an attractive simplicity:

$$\left(\frac{R_{F,B}}{R_{F,A}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}}\right)_2 \left(\frac{X_{S,2}}{X_{S,1}}\right)^{|\Delta m|} \tag{8}$$

Snyder. Precisely the same line of reasoning can be applied to the work of Snyder, with effectively the same result (Snyder has already shown that his and Soczewinski and co-workers' models have the same form[19]). Snyder states[20], "R_F values for two thin-layer systems differing only in the solvent can be related as

$$(R_M')_1 - (R_M')_2 = \alpha A_S(\varepsilon_2 - \varepsilon_1) \tag{9}$$

$(R_M')_1$ and $(R_M')_2$ refer to R_M' values for a given sample and adsorbent, for solvents 1 and 2, respectively". (Our eqn. 9 is Snyder's eqn. 8-5a.)

In eqn. 9, α expresses the adsorbent activity parameter[21] and A_S the molecular area of the adsorbed polar component S[22]. It will be convenient for us to express the product αA_S as a constant, c.

R'_M differs from R_M as follows. Whereas R_M is $\ln[(1-R_F)/R_F]$, R'_M is $\ln[(1-\xi R_F)/\xi R_F]$. Snyder comments[23]: "ξ is essentially independent of R_F for a given adsorbent and solvent, except that it approaches a value of 1.0 for very weakly adsorbed samples... In most thin-layer chromatography operations ξ is equal to about 1.1". Our work concerns those relatively strongly adsorbed samples for which $(1-\xi R_F)$ approaches unity, and R_F ratios for a given adsorbent and solvent for which $(\xi R_F)_2/(\xi R_F)_1 = R_{F,2}/R_{F,1}$. For both reasons, we shall neglect ξ.

We restate eqn. 9 for our substances A and B, expressing $\varepsilon_2 - \varepsilon_1$ as $\Delta\varepsilon$:

$$(R_{M,A})_1 - (R_{M,A})_2 = c_A \Delta\varepsilon \tag{10}$$

$$(R_{M,B})_1 - (R_{M,B})_2 = c_B \Delta\varepsilon \tag{11}$$

We subtract eqn. 11 from eqn. 10:

$$(R_{M,A} - R_{M,B})_1 = (R_{M,A} - R_{M,B})_2 + \Delta c \, \Delta\varepsilon \tag{12}$$

where $\Delta c = c_A - c_B$. Re-expressing eqn. 12 in terms of R_F, we have

$$\left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1-R_{F,A}}{1-R_{F,B}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1-R_{F,B}}{1-R_{F,B}}\right)_2 e^{\Delta c \Delta\varepsilon} \tag{13}$$

Equivalence of expressions. We wish to relate eqns. 7 and 13. We shall do this by re-expressing eqn. 7 in terms of the solvent strength, ε. Eqn. 7 deals with the Soczewinski binary mixtures each of which contains cyclohexane and a molar fraction X_S of a polar solvent S. With a given adsorbent, a given less polar component (here, cyclohexane) of solvent strength ε_0, and a given more polar component, the solvent strengths ε_1 and ε_2 of the binary mixtures 1 and 2 are approximately equal to $[\varepsilon_0 + (1/c)(\log X_{S,1})]$ and $[\varepsilon_0 + (1/c)(\log X_{S,2})]$, respectively[24]. The solvent strength difference between them is $[(1/c)\log(X_{S,1})/(X_{S,2})]$. Therefore,

$$\log\left(\frac{X_{S,1}}{X_{S,2}}\right)^{|\Delta m|} = \Delta\mu\Delta\varepsilon \tag{14}$$

where $\Delta\mu$ expresses the product $c\,\Delta m$. As noted earlier, the constant c (ref. 24) expresses the product of the adsorbent activity parameter, α, (ref. 21) and the molecular area, A_S, of the adsorbed polar component S (ref. 22). Both are positive. These are included in the slope m but excluded from the solvent strength, ε. Therefore the expressions that explicitly state the solvent strength will have the slope difference $\Delta\mu$ rather than Δm. Because both the slope difference, $\Delta\mu$, and the solvent strength decrease, $\Delta\varepsilon$, are negative, the product $\Delta\mu\Delta\varepsilon$ is positive, and absolute magnitude signs can be omitted.

We substitute eqn. 14 into eqn. 7:

$$\left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1-R_{F,A}}{1-R_{F,B}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1-R_{F,A}}{1-R_{F,B}}\right)_2 \exp \Delta\mu\Delta\varepsilon \tag{15}$$

Eqn. 15, which is a re-expression of eqn. 7, has the same form as eqn. 13. Eqns. 15 and 7 differ from eqn. 13 only by the explicit reference in eqn. 13 to the

adsorbent activity parameter, α, and the molecular area, A_S, on the adsorbent of the polar component S of the solvent. Because both α and A_S are constants for a given solute, adsorbent and solvent polar component, eqns. 15 and 7 may be taken as equivalent to eqn. 13 for the systems we are considering.

(b) Further comments

The selectivity equations: theoretical basis and experimental proof. Eqns. 7, 13 and 15, which we shall refer to as the selectivity equations, are logical consequences of eqns. 1 and 9.

Eqns. 1 and 9, which Snyder[19] has pointed out are equivalent in form, correlate the many observations of Soczewinski and co-workers and Snyder, respectively, on liquid–solid adsorption. Those observations cover hundreds of compounds of numerous types.

The selectivity equations merely express explicitly what is already implicit in eqns. 1 and 9; they may be expected to hold over the same experimental ranges. Accordingly, the experimental observations to be described illustrate but do not and need not "prove" the selectivity equations. Soczewinski and co-workers and Snyder have already supplied that proof in abundance, albeit for their own purposes.

Exceptions. Eqns. 1 and 9 suggest a straight-line behavior. Such behavior is usual but not universal. With some compounds, the slope of the plot that describes their behavior changes abruptly at low solvent strengths as the R_F ceases to diminish at the same rate with further decrease of solvent strength. The behavior of these compounds is described by two straight-line segments joined at a low-solvent-strength knee. More rarely, compound behavior is described by a curve. These exceptions make increasing selectivity by decreasing solvent strength occasionally less predictable, but not less useful.

Degree of selectivity increase at high solvent dilution. Eqns. 1 and 9 come from studies that were not carried to very low solvent strengths compared with the strength of the starting solvent. Thus the effects predicted by eqns. 7 and 15 may or may not be found at high dilutions; that area simply has not been investigated. For practical purposes the question tends to be academic, since the selectivity increase as the polar solvent is diluted is usually quickly observable.

Eqn. 17 suggests, in accordance with the suggestion of Thoma[18], that if the more polar component of the binary solvent has some larger solvent strength (0.50, for instance) that is different from zero and the diluting component has zero solvent strength, then the selectivity can be increased without limit. This, of course, requires that the substances A and B show non-identical behavior that remains linear as the solvent strength of the binary approaches zero. In very limited testing, we have not as yet observed a realization of this intriguing possibility.

Intersectional behavior. Because identical behavior between two molecular species is neither usual nor expected, the straight lines showing such behavior almost always show different slopes and, not infrequently, may intersect. That intersection may occur at almost any solvent strength, we have found (see, for instance, Fig. 8-1 in Snyder's book[14]). At the intersection, the two compounds cannot be separated, and then either higher or lower solvent strengths improve the separation. If the intersection does not occur at what is already too high a solvent strength, then a higher solvent strength speeds as well as improves the separation. However, increasing solvent

strength is an obviously limited way of increasing selectivity: the compounds get washed into the solvent front. In contrast, so long as the behavior remains linear, decreasing solvent strength to improve selectivity is not limited: R_F values diminish but never become zero. Indeed, decreasing the solvent strength can take a poor separation back through an intersection and then improve it as desired. Oil Orange shows this behavior very clearly with Sudan Green: see the R_F ratios in Table 2, and also Figs. 1 and 2. The Oil Orange–Sudan Green intersectional behavior is even better shown with acetone–hexane mixtures as the developing solvents[25]. See also the next section, wherein an example cited by Snyder[15] for selectivity enhancement is shown to display intersectional behavior as well.

B. Selectivity: definition, measurement and enhancement: a dialogue

(a) Definition and measurement

Snyder. "Selectivity (may be defined as) the ability of a system to separate the centers of bands 1 and 2"[16].

Reply. We would prefer, in order completely to divorce selectivity from bed efficiency, length and use, to define selectivity as the *eventual* ability of a system to separate the centers of bands 1 and 2.

Snyder. The "ratio K_1/K_2 (may also be referred) to as the selectivity of the adsorption system..."[16].

Reply. Relative motion manifests selectivity: band 1 must move faster or slower than band 2. But relative motion is the ratio of one velocity to another. In TLC, that ratio is identically the R_F ratio. In TLC selectivity is perhaps best defined as it is manifested and measured: by the R_F ratio.

The ratio K_1/K_2 of distribution coefficient is related to the R_F ratio as follows:

$$\frac{K_1}{K_2} = \frac{(1 - R_{F,1})/R_{F,1}}{(1 - R_{F,2})/R_{F,2}} \tag{16}$$

The R_F ratio therefore differs from the inverse of the distribution coefficient ratio K_1/K_2 only by a multiplier that approaches unity at low R_F values:

$$\frac{R_{F,2}}{R_{F,1}} = \frac{K_1}{K_2} \cdot \frac{1 - R_{F,2}}{1 - R_{F,1}} \tag{17}$$

It can be seen that the R_F ratio provides an increasingly accurate method of measuring the distribution coefficient ratio K_1/K_2 as solvent strengths decrease. (This is increasingly difficult to do by high-performance liquid chromatography because the bands become undetectable in the mobile phase, but increasingly easy by TLC with the short-bed continuous development we shall be discussing.)

(b) Selectivity change with solvent strength

Snyder. "We assume that selectivity (and thus the 'ratio K_1/K_2, which we ... refer to as the selectivity'[16]) remain(s) unchanged with varying sample K values"[17] (and therefore with variations in solvent strength[21]).

Reply. Consider Snyder's eqn. 8-4, given here as our eqn. 18, for a given solute at two solvent strengths[26]:

$$\ln (K_1/K_2) = \alpha A_S (\varepsilon_2 - \varepsilon_1) \tag{18}$$

For our spots A and B, we have from eqn. 18

$$(\ln K_A)_1 - (\ln K_A)_2 = \alpha A_A (\varepsilon_2 - \varepsilon_1) \tag{19}$$

$$(\ln K_B)_1 - (\ln K_B)_2 = \alpha A_B (\varepsilon_2 - \varepsilon_1) \tag{20}$$

We subtract eqn. 20 from eqn. 19, rearrange, and raise to the power of the logarithm base:

$$\left(\frac{K_A}{K_B}\right)_1 = \left(\frac{K_A}{K_B}\right)_2 \exp \alpha \Delta A \Delta \varepsilon \tag{21}$$

where $\Delta A = A_A - A_B$ and $\Delta \varepsilon = \varepsilon_2 - \varepsilon_1$.

Eqn. 21 directly contradicts the assumption that the ratio K_1/K_2, which Snyder equated with selectivity, remains unchanged with "varying sample K values", *i.e.*, with changes in solvent strength. Also, to the extent that eqn. 18 is valid, eqn. 21 also indicates that no change in either A_A or A_B is required for the ratio of distribution coefficients to be an exponential function of solvent strength. (This is consistent with our earlier development. Eqn. 15 can equally well be derived from eqn. 21.)

Snyder. Eqn. 18 "suggests the relative K^0 values of two sample components can be changed by a simple change in solvent strength, whenever the A_S values of the two compounds are different"[15]. (An example is then cited, the separation of 1,2,4,5-dibenzpyrene from 2,6-dimethylpyridine, that strikingly illustrates the very effect that is a principal subject of the present paper.)

Reply. The example also illustrates intersectional behavior. In the example, the selectivity is not only enhanced by a solvent strength decrease from 0.32 (benzene) to 0.00 (pentane), but also *reversed*. Thus two compounds of even widely different A_S values can, under certain circumstances, show *identical* R_F values. Conversely, identical R_F values imply neither highly similar A_S values nor any inapplicability of selectivity enhancement by change in solvent strength.

Also, in the example cited by Snyder, the magnitudes of the A_S value ratios and of the selectivities at the two solvent strengths are of prime interest for us. The A_S values are 15 and 8 for 1,2,4,5-dibenzpyrene and 2,6-dimethylpyridine, respectively, and the corresponding selectivities (measured as relative retention volumes) are 3.3 and 7.9 for benzene and pentane, respectively. Such gross selectivities do not remotely tap the capabilities of TLC. Thus Snyder's qualification, "...whenever the A_S values of the two compounds are different", is far less restrictive than it seems. His overall comment on eqn. 18 might better read, "This suggests the relative K^0 values of two sample components can be usefully changed by a simple change in solvent strength unless (as is unlikely) the A_S values of the two compounds are truly identical".

Snyder. Changing solvent strength to enhance selectivity is "limited in its practical usefulness" for two reasons. The first objection (we present the second in the next exchange) is that "Overlapping sample bands most often occur for compounds of closely related chemical structure... This implies similar or equal A_S values for the two compounds, in which case changes in solvent strength are of no use for the alteration of relative K^0 values"[15].

Reply. Whether all sample bands overlap because of adventitious reasons such as sample complexity or only because of chemical similarity seems to demand too

much insight into the nature of all samples past, present and future. As we have just commented, however, unless the A_S values of two compounds are truly identical, a simple change in solvent strength can usually separate them. A number of chemically similar compounds that do respond as we suggest are cited in section D.

Snyder. The second objection: "Another limitation of (changing solvent strength) for splitting unresolved sample bands is that optimum separation generally occurs for a narrow range of K^0 values corresponding to a solvent of given strength. If two sample compounds of differing A_S values are unseparated using a solvent of this desired strength, they can be separated using a solvent of quite different strength. But this then means that the K^0 values of the two sample components will be either too large or too small for optimum separation. In the case of thin-layer chromatography this is a particularly severe limitation"[15].

Reply. We have already shown, most recently by eqn. 21, that that assumption is false that implies that only a "narrow range of K^0 values corresponding to a solvent of given strength" is acceptable. As Soczewinski and co-workers concluded and as the examples to be cited suggest, separations generally improve sharply with decreasing solvent strength. The experimental technique needed to make this approach feasible is continuous development through a short bed, which technique was developed during and for this work. Note that TLC is the only chromatographic form that is well suited to this approach. Only in TLC are the separated components detected on the stationary phase and thus maximally rather than minimally concentrated in space.

(c) Personal comments by L. R. Snyder

Since a *dialogue* implies the exchange, rebuttal and re-rebuttal of ideas and information, I have offered the following remarks to clarify some of the points raised above.

Firstly, it is stated that eqn. 21 "directly contradicts" the assumption in ref. 16 that K_1/K_2 is constant. I argue in that reference that *for the usual case* where a sample consists of related compounds, and where for some mobile phase composition $K_1 = K_2$, that then $A_1 \approx A_2$. Eqn. 21 then *confirms* the assumption that K_1/K_2 will usually remain constant as solvent strength is varied.

Secondly, after arguing earlier that fan-shaped R_M–X_S plots are generally the rule, the author examines so-called "intersectional behavior" in detail. The conclusion seems to be that a change in solvent strength will almost always lead to a significant change in K_1/K_2 and to separation of a band pair. The author cannot have it both ways for the *general* case: he must either anticipate fan-shaped plots generally, or not. I personally believe that in most cases, fan-shaped plots will be found for samples which contain compounds of related structure. Now for the case of such plots, it is apparent that the closer the ratio K_1/K_2 is to unity for some pair of compounds and for some solvent composition, the *less* likely it is to expect that a change in solvent strength will lead to a significant change in K_1/K_2. Therefore, in the usual case, I believe that a change in solvent strength will be less effective in creating separation selectivity than will a change to a second polar solvent to replace the first; *e.g.*, change from hexane–chloroform to hexane–diethyl ether. Some rules for guiding this change in solvent are reviewed in ref. 27.

Finally, my comments in ref. 16 concerning the application of solvent strength changes in TLC for improved selectivity (as quoted above) apply mainly to conven-

tional TLC without continuous development. When continuous development is used, the *occasional* improvement in selectivity that accompanies the use of lower strength mobile phases will then contribute in a useful way to the further separation of the sample (in addition to increasing resolution through increase in K). Thus, I feel that the present recommendations for taking advantage of lower solvent strengths plus continuous development in TLC should not be overlooked as one more way of achieving the separation of difficult samples. Whether this is preferable in a given instance, particularly considering the other options available for improving selectivity in TLC, must be determined by the user.

C. Quantitative prediction of selectivity increase

The quantitative prediction of selectivity increase tends to be of only academic interest, for several reasons. Exact prediction requires that the R_F *versus* solvent strength behavior be known. This in turn requires rather tedious measurements of R_F at low R_F values. But most important is that the analyst needs not a prediction but a separation. A few short-bed continuous developments at increasing dilutions of the polar component usually quickly produce the separation and bracket its parameters, so prediction is neither needed nor practically useful.

Nevertheless, it is of interest to show that these separations can be described in numbers. The full quantitative description by eqn. 7 or 15 would be exactly as precise as eqn. 1 or 9 that they correlate, and thus of little interest here. But what of the possible omission of the $(1 - R_{F,A})/(1 - R_{F,B})$ multipliers?

We can measure relatively easily the R_F values we get with the undiluted and slightly diluted (say, 1:1 or 2:1) polar component. We wish to predict the R_F ratios we will obtain for further dilutions of the polar component.

Whether we wish to ignore the $(1 - R_{F,A})/(1 - R_{F,B})$ multiplier in the expression for the stronger solvent depends somewhat on the initial difficulty of the separation. With a difficult separation, $R_{F,B}$ is initially almost equal to $R_{F,A}$. Then the multiplier is almost unity itself, and could be omitted. But for R_F values that are initially mutually different, the multiplier should be kept in.

So we shall state and test both simplified forms of eqn. 15:

$$\left(\frac{R_{F,B}}{R_{F,A}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}} \cdot \frac{1 - R_{F,A}}{1 - R_{F,B}}\right)_2 \exp \Delta\mu\Delta\varepsilon \tag{22}$$

$$\left(\frac{R_{F,B}}{R_{F,A}}\right)_1 = \left(\frac{R_{F,B}}{R_{F,A}}\right)_2 \exp \Delta\mu\Delta\varepsilon \tag{23}$$

The quantitative prediction of the R_F ratios to be obtained at lower solvent strengths is tested in Tables 2 and 3. In general, the simpler eqn. 23 is seen to be usable (as, therefore, would be eqn. 8), although the more elaborate eqn. 2 is obviously more accurate.

There is no question that the R_F ratios do change in a quantitatively describable fashion. Eqns. 8 and 23 express this change in an easily understandable format.

The photographs shown in Fig. 1 illustrate the process of selectivity increase by solvent strength decrease. The approach is extremely effective: the extraordinary increase in selectivity shown in these photographs corresponds to a solvent strength

TABLE 2

R_F RATIOS *VERSUS* SOLVENT STRENGTH

Solvent strength	*R**	*E23***	*E22****	*C*§	*R**	*E23***	*E22****	*C*§	*R**	*E23***	*E22****	*C*§
	Butter Yellow–Sudan Green				*Oil Orange–Butter Yellow*				*Oil Orange–Sudan Green*			
0.40	1.0	1.0	1.1	0.9	0.9	0.8	0.8	1.0	0.9	0.8	0.9	0.9
0.35	1.1	1.2	1.2	0.9	1.1	1.1	1.1	1.3	1.2	1.2	1.3	1.2
0.30	1.3	1.4	1.4	1.0	1.4	1.4	1.4	1.6	1.9	1.9	2.0	1.6
0.25	1.5	1.6	1.7	1.1	2.0	1.9	1.9	1.6	2.9	2.9	3.0	1.7
0.20	1.7	1.9	1.9	1.3	2.7	2.4	2.5	2.3	4.5	4.4	4.6	2.9
0.18	1.8	1.7	1.8	1.3	3.0	2.7	3.0	3.1	5.3	5.2	5.5	4.0
	Oil Orange–Sudan III				*Butter Yellow–Sudan III*				*Sudan III–Sudan Green*			
0.40	2.1	2.2	2.9	2.0	2.5	2.5	3.5	2.0	0.42	0.42	0.30	0.45
0.35	3.3	3.0	3.9	3.0	3.0	2.6	3.6	2.4	0.38	0.45	0.34	0.40
0.30	4.7	4.1	5.2	3.7	3.3	2.7	3.7	2.4	0.40	0.53	0.38	0.43
0.25	6.7	5.5	7.1	4.9	3.4	2.8	3.9	2.8	0.43	0.59	0.43	0.40
0.20	9.4	7.5	9.7	5.6	3.5	2.9	4.0	2.5	0.48	0.67	0.47	0.50
0.18	10	8.5	11	7.1	3.5	2.9	4.0	2.3	0.50	0.67	0.50	0.56

* R = Calculated from regression lines.
** E23 = Predicted by eqn. 23, which is the simplest.
*** E22 = Predicted by eqn. 22.
§ C = Observed from continuous developments.

TABLE 3

R_F RATIO FACTORS OF INCREASE *VERSUS* SOLVENT STRENGTH DECREASE

$\Delta\varepsilon$*	*R***	*E23****	*E22*§	*C*§§	*R***	*E23****	*E22*§	*C*§§	*R***	*E23****	*E22*§	*C*§§
	Butter Yellow–Sudan Green				*Oil Orange–Butter Yellow*				*Oil Orange–Sudan Green*			
0.00	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0	0.9	0.9	1.0
−0.05	1.1	1.2	1.2	1.0	1.2	1.2	1.2	1.3	1.4	1.4	1.4	1.3
−0.10	1.3	1.3	1.4	1.1	1.6	1.6	1.6	1.6	2.1	2.1	2.2	1.7
−0.15	1.4	1.6	1.6	1.1	2.2	2.1	2.2	1.6	3.2	3.2	3.3	1.9
−0.20	1.6	1.8	1.8	1.4	3.1	2.8	2.8	2.3	4.9	4.8	5.1	3.2
−0.22	1.7	1.7	1.7	1.5	3.4	3.1	3.2	3.0	5.8	5.7	6.0	4.4
	Oil Orange–Sudan III				*Butter Yellow–Sudan III*				*Sudan III–Sudan Green*			
0.00	1.0	1.0	1.3	1.0	1.0	1.0	1.4	1.0	1.0	1.0	0.71	1.1
−0.05	1.5	1.4	1.7	1.5	1.2	1.0	1.4	1.2	0.90	1.1	0.81	0.95
−0.10	2.1	1.8	2.4	1.8	1.3	1.1	1.5	1.2	0.95	1.3	0.90	1.0
−0.15	3.0	2.5	3.2	2.4	1.4	1.1	1.6	1.4	1.0	1.4	1.0	0.95
−0.20	4.2	3.4	4.3	2.8	1.4	1.1	1.6	1.2	1.1	1.6	1.1	1.2
−0.22	4.7	3.8	4.9	3.5	1.4	1.1	1.6	1.1	1.2	1.6	1.2	1.3

* $\Delta\varepsilon$ = Solvent strength decrease from 0.40.
** R = (Corresponding Table 2 regression-line R_F ratio)/(regression-line ratio for $\varepsilon = 0.40$).
*** E23 = (Corresponding Table 2 eqn. 23 R_F ratio)/(regression-line R_F ratio for $\varepsilon = 0.40$).
§ E22 = (Corresponding Table 2 eqn. 22 R_F ratio)/(regression-line R_F ratio for $\varepsilon = 0.40$).
§§ C = (Corresponding Table 2 continuous development R_F ratio)/(continuous development R_F ratio for $\varepsilon = 0.40$).

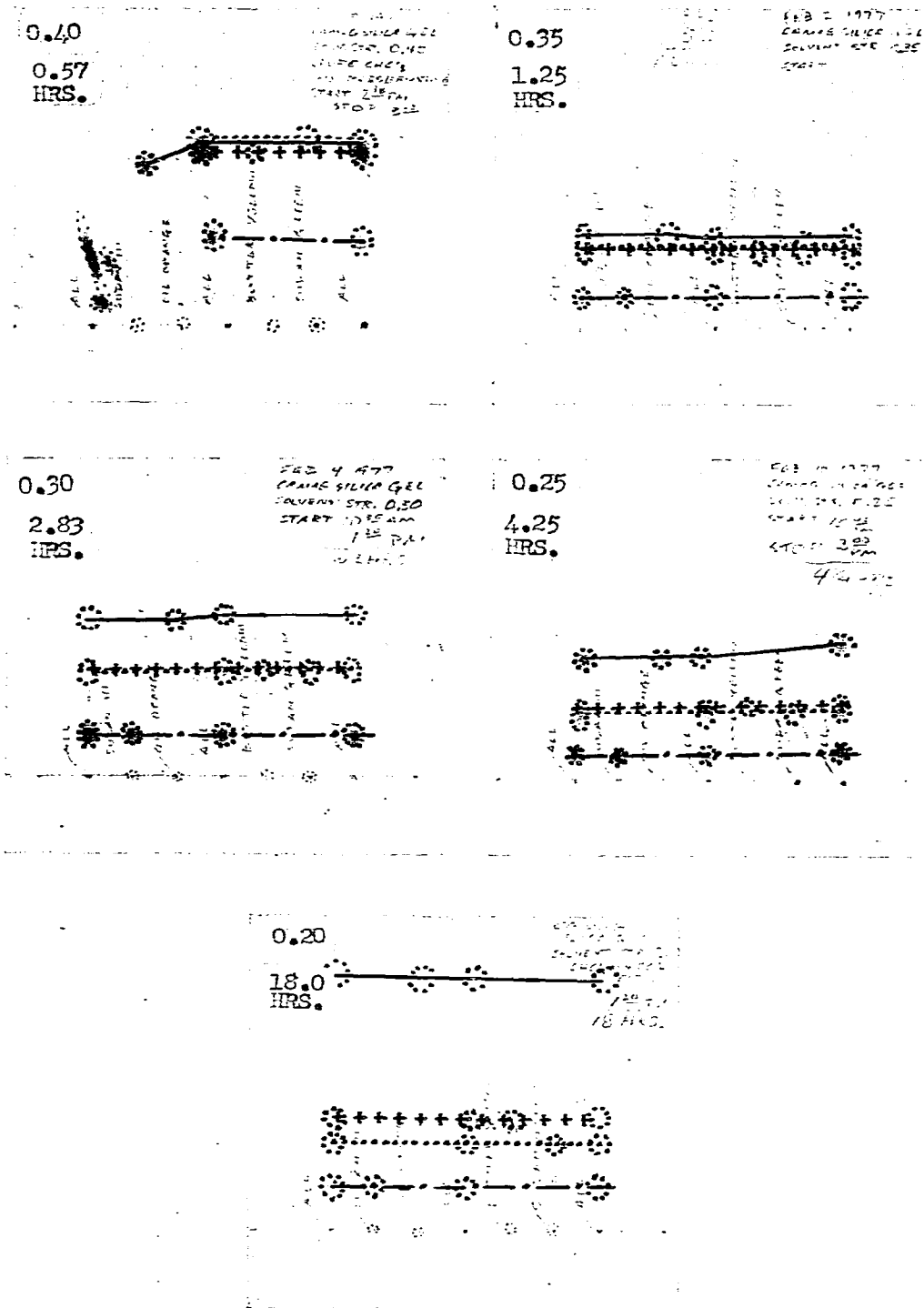

Fig. 1. As solvent strength is reduced, R ratios increase exponentially. Resolution follows easily, while sensitivity remains approximately constant. ——, Oil Orange; ++++, Butter Yellow; ....., Sudan Green; — · ——·, Sudan III.

decrease of only 0.20, from 0.40 to 0.20 in a series of chloroform–carbon tetrachloride mixtures.

D. Further examples

(a) Dilution with cyclohexane (Fig. 2)

Fig. 2 differs from Fig. 1 in several ways. The dyes are the same, but for better visibility the dye concentrations are greater. Also, the solvent strength was decreased by simple dilution with hexane. Thus, in Fig. 2 there is no possible special selectivity being brought to bear, but only that selectivity that is enhanced as the polar solvent is diluted.

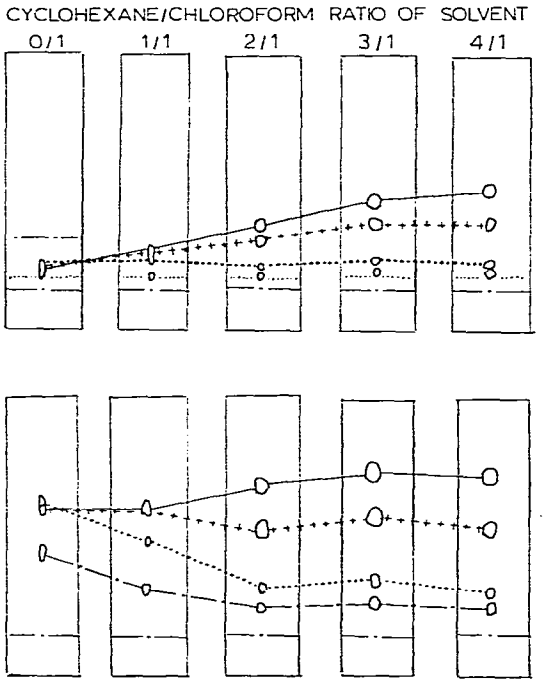

Fig. 2. Simple dilution with cyclohexane usually quickly improves selectivity. The top three dyes show intersectional behavior; an initial decrease in selectivity, followed by an increase on further dilution of the solvent. Dyes: Oil Orange (———); Butter Yellow (+++); Sudan Green (....); Sudan III (—.—.—). Layer: silica gel G.

Fig. 2 also corresponds more closely to normal operations than Fig. 1, with regard to dilutions. In Fig. 2, the solvent that would be used for a conventional development (here chloroform) is first used at full strength and then used in successive dilutions (1:1 to 1:4), with no particular regard for the numerical expression of solvent strength. The top row of plates, in which the extent of development was normalized on a 5-mm advance for the slowest spot (Sudan III), shows clearly the rapid increase in selectivity with increasing dilution of the solvent. The bottom row shows the corresponding plates with development continued until the chromatograms were roughly centered on the bed.

Fig. 2 also shows intersectional behavior, with intersections at various solvent

strengths. In the course of the dilutions, Sudan Green crosses both Oil Orange and Butter Yellow, and apparently would cross that of Sudan III at still greater dilution (see the R_F ratio predictions in Table 2). In any event, a 1:1 dilution causes Sudan Green to cross the Oil Orange–Butter Yellow pair and become cleanly separated from them. The 2:1 dilution increases that separation and also separates all the dyes. The sensitivity advantage that accrues with higher selectivity and developments near the origin can also be seen by comparing the middle plate in the top row with any of the right three plates in the bottom row. This advantage is treated further below.

(b) Sixty examples from the literature

To be usable in practice, selectivity enhancement by solvent strength decrease normally requires the short-bed continuous development technique used here and described below. Thus at the time of writing one should not really expect to find illustrations of the approach already in the literature. Nevertheless, numerous examples of increased selectivity from decreased solvent strength can be gleaned from the second edition of Stahl's book[12]. These include 8 pairs from among 7 fat-soluble vitamins (Table 4), 15 pairs from among 9 tropane alkaloids (Table 5), 9 pairs from among 10 *Vinca* alkaloids (Table 6), 9 pairs from among 5 antidepressives (Table 7), 9 pairs from among 10 phenols (Table 8), and 11 pairs from among 16 phenol derivatives (Table 9). Of these 61 pairs, almost half (27) show an initial R_F ratio of unity: the separations are impossible at normal solvent strengths on the layers in question and with the solvent shown. With solvent strength reduced, however, usually merely by dilution with cyclohexane, the R_F ratios show that the components can then be separated easily.

TABLE 4

INCREASED SELECTIVITY FROM INCREASED CYCLOHEXANE TO DIETHYL ETHER RATIO (C:DE): EXAMPLES FROM THE FAT-SOLUBLE VITAMINS

Assorted pairs from seven vitamins	*R_F ratios**		*Corresponding requirements for theoretical plates*	
	*C:DE = 1:1***	*C:DE = 4:1****	*C:DE = 1:1***	*C:DE = 4:1****
β-Carotene–vit. A palmitate	1.0	1.2	Infinite	600
Vit. A acetate–α-tocopherol	1.1	1.4	2500	180
β-Carotene–vit. K_1	1.1	1.4	2500	180
Vit. K_1–α-tocopherol	1.2	1.9	600	50
Vit. A palmitate–vit. A acetate	1.2	1.6	600	100
α-Tocopherol–vit. D_2 or D_3	1.7	2.1	80	40
Vit. A acetate–vit. A alcohol	2.1	4.5	80	10
β-Carotene–vit. A alcohol	2.6	8.0	25	Negligible

* Source: columns 1 and 2, Table 38, ref. 28. Layer: silica gel G.
** Cited: our ref. 29.
*** Cited: our ref. 30.

In the tables from which these examples were gleaned are other examples in which the solvent strength reduction either decreases or does not affect selectivity. This is consistent with our experience and that of Soczewinski and co-workers: reducing solvent strength usually increases selectivity, but not always.

TABLE 5

INCREASED SELECTIVITY FROM DECREASED SOLVENT STRENGTH IN TWO BASIC SOLVENTS: EXAMPLES FROM THE TROPANE ALKALOIDS

Assorted pairs from nine tropane alkaloids	*R_F ratios**		*Corresponding requirements for theoretical plates*	
	*$CHCl_3$–Et_2NH (9:1)***	*C_6H_{12}–$CHCl_3$–Et_2NH (5:4:1)****	*$CHCl_3$–Et_2NH (9:1)****	*C_6H_{12}–$CHCl_3$–Et_2NH (5:4:1)****
Scopolamine–scopoline	1.0	1.5	Infinite	140
Cocaine–tropacocaine	1.0	1.2	Infinite	600
Tropacocaine–scopoline	1.0	1.3	Infinite	300
New psicaine–tropacocaine	1.0	1.1	Infinite	2500
Cocaine–new psicaine	1.0	1.1	Infinite	2500
Cocaine–scopoline	1.0	1.5	Infinite	140
Apoatropine–scopolamine	1.1	2.1	2500	40
Cocaine–apoatropine	1.3	1.6	300	100
Scopoline–scopolamine	1.5	2.3	140	30
Cocaine–scopolamine	1.5	3.4	140	14
Apoatropine–homatropine	1.5	2.7	140	22
Apoatropine–atropine	1.7	2.5	80	26
Cocaine–homatropine	2.0	4.3	45	10
Cocaine–atropine	2.3	4.1	30	12
Scopoline–atropine	2.3	2.8	30	18

* Source: columns II and III, Table 75, ref. 31, citing our ref. 32. Layer: silica gel G.
** Solvents.

TABLE 6

INCREASED SELECTIVITY FROM DECREASED SOLVENT STRENGTH: EXAMPLES FROM THE *VINCA* ALKALOIDS

Assorted pairs of 10 Vinca alkaloids	*R_F ratios**		*Corresponding requirements for theoretical plates*	
	EtOAc–EtOH (3:1)	*EtOAc (pure)***	*EtOAc–EtOH (3:1)***	*EtOAc (pure)****
Tetrahydroalstonine–catharanthine	1.0	1.8	Infinite	55
Catharanthine–carosidine	1.0	3.8	Infinite	13
Catharanthine–catharosine	1.0	4.8	Infinite	Negligible
Vincarodine–pleurosine	1.0	3.3	Infinite	15
Vindolinine–lochnerine	1.1	2.2	2500	35
Virosine–pleurosine	1.1	3.0	2500	25
Catharanthine–virosine	1.1	4.2	2500	11
Tetrahydroalstonine–ajmalicine	1.1	1.3	2500	300
Catharanthine–catharine	1.1	3.8	2500	13

* Source: columns IIS and IVS, Table 83, ref. 33. Layer: silica gel G.
** Solvents.

E. Thin-layer chromatography at high selectivity

(a) Theoretical plates

Increasing selectivity radically decreases the number of theoretical plates necessary for a given separation[39]. For an R_F ratio of 1.015 and normal R_F values of

TABLE 7

INCREASED SELECTIVITY FROM DECREASED SOLVENT STRENGTH IN TWO BASIC SOLVENTS: EXAMPLES FROM SOME ANTIDEPRESSIVES

Assorted pairs among five antidepressives	R_F *ratios* *		*Corresponding requirements for theoretical plates*	
	$CHCl_3$–Me_2CO–Et_2NH (5:4:1) **	$CHCl_3$–C_6H_{12}–Et_2NH (5:4:1) **	$CHCl_3$–Me_2CO–Et_2NH (5:4:1) **	$CHCl_3$–C_6H_{12}–Et_2NH (5:5:1) **
Tervasid–isocarboxazide	1.0	1.2	Infinite	600
Penelzine–tervasid	1.0	1.1	Infinite	2500
Phenelzine–isocarboxazide	1.0	1.4	Infinite	180
Pheniprazine–phenelzine	1.0	1.1	Infinite	2500
Pheniprazine–tervasid	1.0	1.2	Infinite	600
Pheniprazine–isocarboxazide	1.0	1.2	Infinite	600
Pheniprazine–iproniazide	1.7	10.3	80	Negligible
Tervasid–iproniazide	1.7	8.5	80	Negligible
Phenelzine–iproniazide	1.7	9.5	80	Negligible

* Source: Table 104, ref. 34, citing our ref. 35. Layer: silica gel G.
** Solvents.

TABLE 8

INCREASED SELECTIVITY FROM DECREASED SOLVENT STRENGTH: EXAMPLES FROM SOME PHENOLS

Assorted pairs from 10 phenols	R_F *ratios* *		*Corresponding requirements for theoretical plates*	
	C_6H_6–CH_3OH (95:5) **	C_6H_6 (pure) **	C_6H_6–CH_3OH (95:5) **	C_6H_6 (pure) **
m-Cresol–phenol	1.0	1.1	Infinite	2500
3,5-Dimethylphenol–3,4-dimethylphenol	1.0	1.1	Infinite	2500
o-Cresol–*p*-cresol	1.1	1.3	2500	300
2,5-Dimethylphenol–2,4-dimethylphenol	1.1	1.2	2500	600
2,6-Dimethylphenol–2,5-dimethylphenol	1.2	1.4	600	180
o-Cresol–*m*-cresol	1.2	1.5	600	140
2,3-Dimethylphenol–*p*-cresol	1.2	1.3	600	300
2,3-Dimethylphenol–phenol	1.4	1.6	180	100

* Source: Table 149, ref. 36, quoting our ref. 37. Layer: silica gel G.
** Solvents.

about 0.4, roughly 100,000 theoretical plates would be required. But if the ratio were increased to 1.5, only about 140 plates would be needed; and if $R_{F,B}/R_{F,A}$ were increased to 2.0, then only about 40 theoretical plates would have to be used. As eqns. 8 and 12 suggest, as the evidence in Table 2 confirms, and as the examples in Figs. 1 and 2 and Tables 4–9 demonstrate, such increases in R_F ratio are usually easily available.

TABLE 9

INCREASED SELECTIVITY FROM DECREASED SOLVENT STRENGTH: EXAMPLES FROM SOME PHENOL DERIVATIVES

Assorted pairs from 16 phenol derivatives	R_F *ratios*		*Corresponding requirements for theoretical plates*	
	Benzene–acetone (90:10) **	*Toluene–acetone (95:5)* **	*Benzene–acetone (90:10)* **	*Toluene–acetone (95:5)* **
Ferulic acid acetate–dimethoxycinnamic acid	1.0	2.5	Infinite	26
Anthraquinone–menadione	1.0	1.1	Infinite	2500
Umbelliferone–quercetin pentaacetate	1.0	1.2	Infinite	600
A quercetin ether***–4′-hydroxychalkone	1.0	1.1	Infinite	2500
A cinnamic acid§–umbelliferone	1.0	1.6	Infinite	100
4′-Hydroxychalkone acetate–alkannin	1.0	1.5	Infinite	140
A cinnamic acid§–quercetin pentaacetate	1.0	2.0	Infinite	45
A cinnamic acid§–(+)-catechin pentaacetate	1.0	2.0	Infinite	45
Anthraquinone–benzoquinone	1.1	1.4	2500	180
4′-Hydroxychalkone–kaempferol tetraacetate	1.1	2.3	2500	30
Veratric acid–ferulic acid acetate	2.3	5.8	30	Negligible

* Source: Table 163, ref. 38. Layer: silica gel G.
** Solvents.
*** Quercetin 5,7,3′,4′-tetramethyl ether.
§ 3,4-Methylenedioxycinnamic acid.

(b) Necessary R_F ratio. Resultant resolution

The migration distance that corresponds to 10–20 (or, indeed, 50–100) theoretical plates on a thin-layer plate is negligible, being less than 1 mm (ref. 40). In other words, at high selectivities technique become limiting. How big are the deposited spots? How much overloaded are they? What real migration distance is then required?

The actual migration distance necessary to disengage two spots is determined by the $R_{F,B}/R_{F,A}$ ratio; the diameter, D, of the deposited spots; and the spreading coefficient, S, the increase in spot radius per unit distance of spot travel. If we let X be the distance the solvent travels during the separation, then the actual migration distance, $R_{F,B}X$, of the faster moving spot must equal the sum of three distances. The first is the distance $R_{F,A}X$ that the slower spot has traveled; the second is the diameter, D, of the deposited spot; and the third is the combined increase, $SR_{F,B}X + SR_{F,A}X$, in the radii of the spots. So we have

$$R_{F,B}X = R_{F,A}X + D + SR_{F,B}X + SR_{F,A}X \tag{24}$$

Eqn. 24 can be rearranged to show the R_F ratio necessary to separate two real

spots with a resolution of unity when that ratio can arbitrarily be made high enough to render negligible the theoretical plate requirement:

$$\frac{R_{F,B}}{R_{F,A}} = \frac{1+S}{1-S} + \frac{D}{(1-S)(R_{F,A}X)} \tag{25}$$

Eqn. 24 can also be rearranged to express the relation, R_s, under these circumstances:

$$R_s = \frac{1 - \left(\frac{R_{F,A}}{R_{F,B}}\right)}{\frac{D}{R_{F,B}X} + S\left(1 + \frac{R_{F,A}}{R_{F,B}}\right)} \tag{26}$$

(c) Spot spreading with spots of low R_F

Obviously, the spreading S should be minimized. In practice, most of the spreading observed at low R_F values is caused by overloading.

Overloading aside, however, a highly favorable facet of low-R_F developments is that spreading is primarily a function of migration distance rather than of time. The theoretical background for understanding this was developed by Thoma[41].

Thoma[41] considered the terms that described the causes of spot spreading. All except one of these are multiplied by the spot R_F. These causes for spot spreading therefore decrease exponentially in effectiveness as solvent strength decreases.

The one term that is not multiplied by the R_F expresses diffusivity in the stationary phase. Diffusion is negligible on adsorbent surfaces. Although Thoma did not point it out, we can realize that the surfaces of the separate particles are not in effective contact with each other. Therefore, spreading while in the adsorbed state can at most proceed only to the boundary of a given particle. Concentration gradients that extend over many particles cannot be dissipated by diffusion in the adsorbed state: spot density is conserved insofar as this spreading mechanism is concerned.

In sum, low-R_F spots spread most because they are overloaded. Aside from this, they spread primarily with migration distance, essentially not at all with development duration. With the high selectivities that become available at low solvent strengths, low-R_F spots need not migrate far. Therefore, they spread little and retain most of their initial density and detectability. This advantage increases as overloading decreases, a facet of importance for trace detection.

F. An illustrative example. Practicality of the approach

Consider the Oil Orange–Sudan Green example. The increasing separation of these with decreasing solvent strength has already been shown in Figs. 1 and 2, but in those experiments no attention was paid to time, other than to record it, or to sensitivity.

The two are shown again in Fig. 3. The bottom section portrays the result of a conventional development on a 20-cm bed with chloroform, solvent strength 0.40, as the developing solvent. The R_F values are about 0.38. Only the spots deposited from the unit-concentration solutions can be detected. The spot densities do not reflect the solvent strength of the solvent from which the spot was deposited. The substances have not been separated.

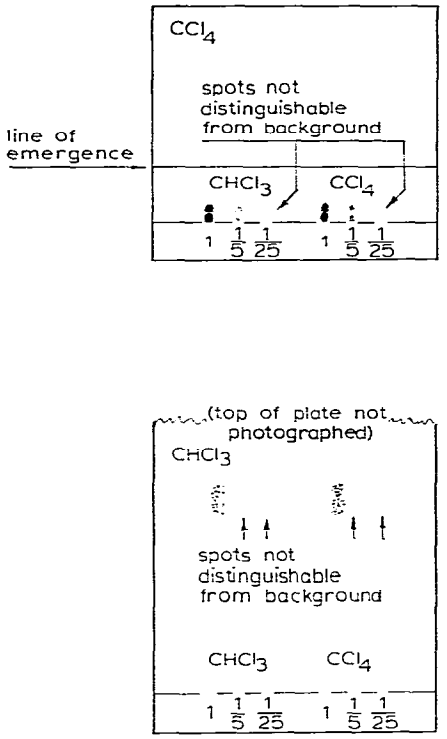

Fig. 3. Representation of a difficult separation. At the bottom is shown a conventional development with conventional R_F values normally considered about optimal. At the top is shown the same separation at a lower solvent strength, with R_F values less than 0.05. The short-bed continuous development shown at top is faster (35 *versus* 90 min) and yields five to ten times better spot detectability.

The top part of Fig. 3 portrays the result of a 35-min continuous development across a 2.5-cm bed with carbon tetrachloride, solvent strength 0.18, as the developing solvent. The R_F values are less than 0.05. (The line of emergence of the plate from inside the development chamber is marked on the plate and is indicated on the figure.) The development was arbitrarily allowed to continue until the spots that had been deposited from chloroform seemed well resolved. The R_F ratio seems to be about 3.5, even with the overloaded spots. The faster moving spot has moved perhaps 5 mm while establishing a center-to-center separation from the slow spot of about 4.5 mm. The resolution varies with the loading and with the solvent from which the spots were deposited.

One can compare the well resolved, low-R_F spots from the 35-min short-bed continuous development with the unresolved conventional-R_F spots from the 90-min conventional development. The low-R_F spots are, in addition to the other advantages, also at least five (possibly ten) times more detectable.

G. Spot deposition

The more detectable and better resolved low-R_F spots in Fig. 3 were deposited

from carbon tetrachloride rather than from chloroform. Fig. 4 shows a further and separate test of spot density as a function of the solvent strength of the spotting solution. The solvent in the original spotting solution should have the lowest solvent strength possible. The components will then tend to be adsorbed at the very point of deposition, and the deposited spot will be as small as the adsorbent bed allows. Accordingly, the low-R_F spots produced by spotting the Oil Orange–Sudan Green mixture from carbon tetrachloride can be seen in Fig. 4 to be five to ten times more detectable than the high-R_F spots produced by spotting from chloroform. The two parts of Fig. 3 show that this initial advantage in detectability is retained near the origin, but largely lost after several centimeters' migration.

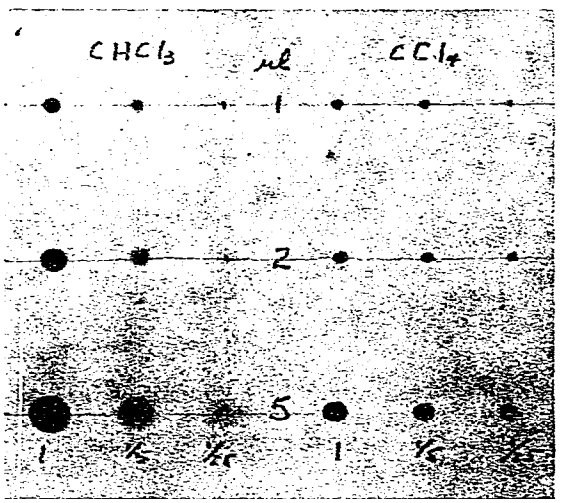

Fig. 4. Spot density *versus* solvent strength. With these dyes, $CHCl_3$ yields R_F values of about 0.4 and CCl_4 about 0.04. The low-R_F spots from CCl_4 are five to ten times as dense as those from $CHCl_3$. The smaller spot diameters also shorten the migration needed for separation.

For the best sensitivity, therefore, as well as for quickest and easiest resolution, spots should be deposited with a minimal initial diameter, then separated at high selectivity with minimal migration.

H. Short-bed versus conventional continuous development

We can at this point profitably compare this use of continuous development with the conventional use. In conventional continuous development, spots are developed across a full 20-cm bed. The emphasis is not on solvent selectivity, presumably already maximized without recourse to solvent strength decrease, but on bed length, that is, on more theoretical plates. But resolution increases only as the square root of the theoretical plate number. In the conventional approach, spot spreading is necessarily maximized both by distance and by the relatively higher R_F values conventionally employed (thus in the literature one sees only conventional continuously developed streaks, never spots: the spots tend to disappear). Finally, the solvent

flow-rates are also necessarily exactly minimal. Thus, conventional continuous development minimizes detectability, takes as long as possible and gains very little in resolution — perhaps, if we wish to be generous, a factor of two, corresponding to four times as many theoretical plates.

In contrast, high-selectivity, low-R_F short-bed continuous development reduces the theoretical plate requirement, usually by whatever factor necessary, essentially to zero; maximized detectability; and takes a minimum of time for the task at hand. If the task is a conventional separation (for which conventional continuous development would not even be considered), then the new approach is superior: faster, with better spot detectability. If the task is an improvement of separation, then conventional continuous development could be used with lower solvent strengths, but it would take longer by the ratio of bed lengths and spot detectability would decrease by a large factor.

4. SUMMARY

Extensive studies of adsorption models show that with the usual solute–solvent–adsorbent system, $\ln [(1 - R_F)/R_F]$ varies linearly with solvent strength. The $\ln [(1 - R_F)/R_F]$ *versus* solvent strength plots of most such systems also diverge mutually with decreasing solvent strength. For the usual systems, then, it can be easily established that selectivity (the center-to-center separation ability of a system) increases exponentially with decrease in solvent strength. The increase can be described simply and quantitatively in terms of R_F.

At the high selectivities that become available with decreased solvent strengths, the number of theoretical plates required for resolution usually becomes negligible. Spots are then resolved after only very short migrations. Spots so resolved remain essentially as detectable as they were at the origin. Such spots are conveniently developed by continuous development across relatively short beds of the adsorbent. The durations of such developments compare favorably with conventional developments.

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