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# THIN-LAYER CHROMATOGRAPHY AS A PILOT TECHNIQUE FOR RAPID COLUMN CHROMATOGRAPHY

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SUMMARY

It is proposed to utilize thin-layer chromatography for the choice of adsorbent, solvent and working conditions in column chromatography (liquid–solid chromatography), as thin-layer chromatography facilitates manipulation, is economical and, unlike column chromatography, enables numerous specific detection reactions to be used. Transfer formulae are developed whose validity is shown by several practical examples.

The transfer of conditions and separations poses few problems, when single-component solvents are used. Under certain conditions (N-chamber), the use of solvent mixtures may lead to uncertainties. The activity of the plate and the column must be controlled and the water concentration of the column chromatographic solvent must be "isotonic" with the column activity. On the other hand, the column activity can be adjusted by using different water concentrations in the solvent.

## INTRODUCTION

Since both thin-layer chromatography (TLC) and column chromatography (CC) are forms of liquid chromatography, separation mechanisms for TLC and CC obviously become identical if the sorbent and solvent are the same. The resolving power of columns in modern, rapid CC is greater than that of the plate[1] whose available number of separation "plates" is limited by the maximum length of the plate (10 or 15 cm); *e.g.*, the maximum number of theoretical plates is approximately 2000 for TLC and 100 000 for CC. Thus, theoretically any separation problem for liquid chromatography can be solved in an optimum way by CC. In many instances, there are nevertheless many reasons for using only TLC, or to use TLC as a pilot technique for CC. The latter instance is the main subject of this paper, which, in addition, deals only with liquid–solid chromatography (LSC). The relative merits of TLC are as follows:

(1) All compounds can be detected visually, often with a selective and specific colour reaction, whilst CC detectors are at present mostly non-specific and, in general, unsatisfying but nevertheless useful for a quantitative approach; there is no com-

pound "irreversibly retained" in a column. TLC therefore gives a good survey of the complete sample.

(2) In TLC, the time required to change a set of conditions (sorbent, solvent) is very short, of the order of half an hour, compared with several hours in CC. Re-equilibration of a column after a change of solvent takes at least 1 h.

(3) Modern separation chambers[2,3] for TLC permit the comparison of several different separation conditions side-by-side on the same plate within about 20 min.

The main advantage of TLC is therefore its great economy in time and materials. Both techniques finally become complementary: TLC is then used to pre-optimize the separation conditions[1,4,5], which cannot be further improved by this technique. Separation of the compounds of interest may be *partial* or *complete*. For partial resolution by TLC, the reason for changing to CC would be to complete the separation. For complete separation, the reason for changing to CC could be either for preparative separation or for easier quantitative handling of the chromatogram. By analogy, with evolution of gas chromatography (GC), one can predict that in future, preparative CC will consist of repetitive injections of "analytical" quantities of the sample into highly efficient narrow columns, rather than the application of large quantities on columns of large diameter (the question of whether it is more advantageous to carry out preparative separations on thick layers or on columns is not considered here).

Of the complementary couple TLC and CC, TLC is the more flexible and dynamic technique, while CC is more static but is highly efficient. In other words, the optimization of the selectivity[5] for a particular separation problem (*i.e.*, the choice of the best combination of sorbent and solvent), is best carried out by TLC; after transferring this selectivity to CC, *i.e.*, by using the same sorbent, solvent and activity, the limited resolution of TLC is brought to a maximum, and parameters such as column length and solvent velocity are optimized. A maximized TLC separation can always be improved by using CC; otherwise, something is wrong. The reasons for failure of such transfers can be as follows: (a) transfer of non-identical systems, *i.e.*, different selectivities, because of differences in sorbent, solvent (and their distribution) and activity; (b) incorrect transfer formulae; or (c) occasional disturbances from the metallic column walls.

## FORMULAE FOR TRANSFER FROM TLC TO CC

When a substance with a certain $R_F$ value is chromatographed in a dry column of length $z$ until the first drop of solvent leaves the column, the substance will have migrated a distance $z_x = R_F \cdot z$. During this time, a volume of solvent $V_m$ enters the column; this is the so-called dead volume. Until elution of the substance ($z_x = z$), a total of $V_{m+s}$ cm$^3$ of solvent must have entered the column:

$$V_{m+s} = \frac{V_m}{R_F} \tag{1}$$

Moreover $V_{m+s} = V_m + V_s$. The term $V_{m+s}$ is the so-called "gross retention volume". Until the moment of elution, in addition to the dead volume, $V_m$, the "net retention volume", $V_s$, has entered the column. Hence, in a dry column, the net retention volume, $V_s$, is equal to the volume of solvent having left before the appear-

ance of the peak maximum at the end of the column. The whole solvent volume that has entered the column up to this moment is equal to $V_m + V_s$.

Eqn. 1 is equally valid for elution from a wet column. Between the injection and elution of a compound with $R_F = 1$, the dead volume, $V_m$, leaves the column. Every compound with $R_F < 1$ then has a true retention volume, $V_s$, in addition to $V_m$.

A more easily understood description of the situation can be given in terms of the retention times in a flowing system. A sorbate molecule with $R_F = 1$ stays only in the mobile phase. To elute it, the (dead) time*, $t_m$, is required. Sorbates with $R_F < 1$ remain for the additional time, $t_s$, in the stationary phase. Hence their effective retention time is equal to $t_s$. The gross retention time, $t_{m+s}$, (often imprecisely called "retention time") therefore consists of the dead time, $t_m$ (*i.e.*, the time it remains in the mobile phase) and of $t_s$ (the additional time it remains in the stationary phase).

The values of $t_s$, $t_m$ and $t_{m+s}$, and $V_s$, $V_m$ and $V_{m+s}$, are proportional to each other. The proportionality factor is the solvent flow, $F_m$ (cm³/min). $V_s = F_m \cdot t_s$.

The basic equations for $R_F$ in adsorption chromatography are:

$$(R_F')_{TLC} = \frac{1}{1 + (K_G)_{TLC}\,(W_a/V_m)_{TLC}} \tag{2}$$

$$(R_F)_{CC} = \frac{1}{1 + (K_G)_{CC}\,(W_a/V_m)_{CC}} \tag{3}$$

where $K_G$ is the adsorption coefficient, $W_a$ is the weight of the sorbent and $W_a/V_m$ is the "phase ratio". $R_F'$ is a corrected $R_F$ value and is approximately 1.1 times greater than the measured value in an S-chamber, and about 1.5 greater in an N-chamber (for details see ref. 5, p. 42). The essential prerequisite for a successful transfer from TLC to CC is to keep constant the adsorption coefficients of the sample components, *i.e.*, to keep constant their selectivities. Hence:

$$(K_G)_{TLC} = (K_G)_{CC}$$

Combination of eqns. 2 and 3 gives, by analogy with the work of SCHULTZ AND COMBERG[7]:

$$(R_F)_{CC} = \frac{1}{1 + \left[(W_a/V_m)_{CC}\,(W_a/V_m)_{TLC}\left(\frac{1}{(R_F')_{TLC}} - 1\right)\right]} \tag{4}$$

Combination of eqns. 1 and 4 gives:

$$\frac{V_s}{V_m} = \frac{(W_a/V_m)_{CC}}{(W_a/V_m)_{TLC}}\left[\frac{1}{(R_F')_{TLC}} - 1\right] \tag{5}$$

Eqn. 5 relates the measured values of the column, $V_s$ and $V_m$, to the TLC $R_F'$ value. The ratio $V_s/V_m$ is the so-called "capacity factor", $k$, or "partition number". It is a specific value ("Kennzahl") for a particular compound in the same way as the $R_F'$ value. Its logarithm is equal to $R_m$ in TLC. The ratio $V_s/V_m$ is independent of the dimensions and the dead volume of a column.

* Symbols in the A.S.T.M. nomenclature are: $t_R$ for $t_{m+s}$, $t_M$ for $t_m$ and $t'_R$ ("adjusted retention time") for $t_s$. See also ref. 6.

Eqn. 5 allows the calculation of $V_s/V_m$ values from $R_F$ values within an error of $\pm$ 5%. Experimental proof is given later. To use this formula, the phase ratios $W_a/V_m$ and the dead volumes $V_m$ of the column and layer must be known. They may differ very much with the sorbent, grain diameter and the type of column packing. Moreover, the ratio $W_a/V_m$ is somewhat dependent on the activity of the sorbent (see Fig. 2, below).

As the calculation of the transfer coefficient, $k_f$,

$$k_f = \frac{(W_a/V_m)_{CC}}{(W_a/V_m)_{TLC}} \tag{6}$$

is tedious in practice, $k_f$ can be more easily calculated through the relationship:

$$k_f = \frac{(V_s/V_m)_{\text{measured by CC}}}{(V_s/V_m)_{\text{calculated from TLC}}} \tag{7}$$

The experimental values of $V_s/V_m$ are divided by $V_s/V_m$ values calculated from a TLC $R_F$ value via eqn. 8 (eqn. 5 with $k_f = 1$):

$$\frac{V_s}{V_m} = \left[\frac{1}{(R_F')_{TLC}} - 1\right] \tag{8}$$

The determination of $(R_F')_{TLC}$ and $(V_s/V_m)$ for a single substance with $0.2 < R_F < 0.8$ is sufficient to calculate $k_f$. The $R_F$ value of any compound in any mixture can then be converted by using this factor, provided that the chromatographic conditions are kept constant. The coefficient $k_f$ refers to a certain activity and a certain column with its specific packing density. To satisfy more exacting requirements, $k_f$ must be determined for every activity and each new column (even if the sorbent and solvent are the same).

The final formula is then (eqn. 5 simplified):

$$\frac{V_s}{V_m} = k_f\left[\frac{1}{(R_F')_{TLC}} - 1\right] \tag{9}$$

*Practical example*

Anthracene was chromatographed on a TLC plate of Kieselgel 60 (Merck) in an S-chamber with *n*-hexane at 58% relative humidity. An $R_F$ value of 0.30 was obtained, corresponding to $R_F' = 0.33$. Eqn. 8 gives $(V_s/V_m)_{\text{calculated}} = 2.00$. On a column filled with an identical sorbent with the same activity (corresponding to 58% relative humidity; for conditioning see later). Anthracene was eluted with $V_s/V_m = 2.43$. Hence, $k_f$ is calculated from eqn. 7: $k_f = 2.43/2.00 = 1.21$.

The $R_F$ values of a mixture of polyphenyls (for chromatogram, see Fig. 1) obtained on the same TLC system were transformed into $V_s/V_m$ values and compared with experimental values (Table I).

## DEAD VOLUME AND PHASE RATIO IN TLC AND CC

As eqns. 5 and 9 show, the correct measurement of the dead volume, $V_m$, is important for the calculation of $V_s/V_m$. Unlike in TLC, where the ratio between the stationary and mobile phase does not vary much from plate to plate (even for

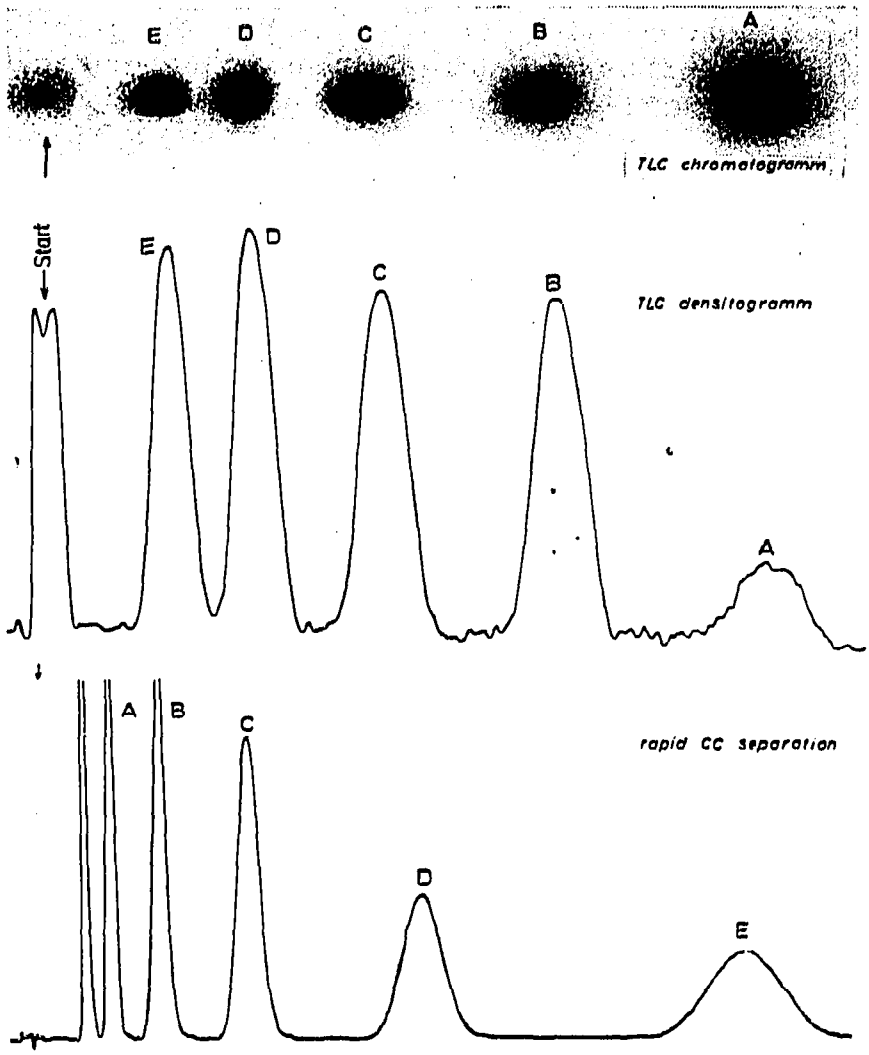



Fig. 1. Comparison of TLC and rapid column chromatography carried out with the same adsorbent (Kieselgel 60, Merck) of different grain diameter but equal activity (58% relative humidity). Solvent, *n*-hexane. Top: TLC chromatogram, single development. Detection by fluorescence quenching. A = biphenyl; B = *m*-terphenyl; C = *m*-quaterphenyl; D = *m*-quinquiphenyl; E = *m*-sexiphenyl; KS-chamber. Middle: densitometer trace of the TLC plate after spraying with nitric acid at a wavelength of 366 $\mu$m. Bottom: CC separation: grain fraction 20–30 $\mu$m; 33 atm. For further details, see text.

TABLE I

COMPARISON OF EXPERIMENTAL AND CALCULATED RETENTION DATA
For technical details, see legend to Fig. 1.

| *Polyphenyl compound* | *Experimental data* | | *Calculated data* |
|---|---|---|---|
| | $(R_F)_{TLC}$ | $V_s/V_m$ *(found)* | $V_s/V_m$ *(calculated from eqn. 9)* |
| Biphenyl | 0.52 | 0.88 | 0.91 |
| *m*-Terphenyl | 0.35 | 1.89 | 1.92 |
| *m*-Quaterphenyl | 0.22 | 3.92 | 3.80 |
| *m*-Quinquiphenyl | 0.13 | 7.83 | 7.25 |
| *m*-Sexiphenyl | 0.07 | 15.10 | 14.50 |

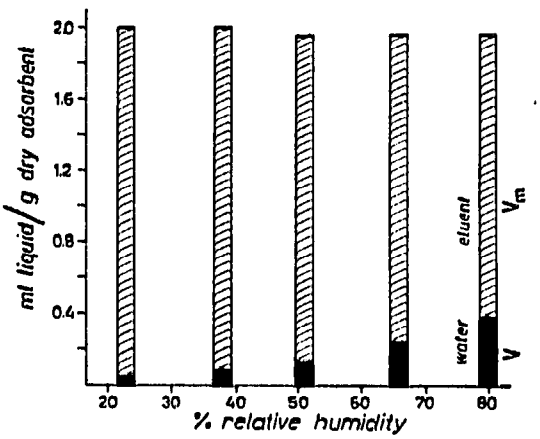



Fig. 2. Decrease of the free layer volume, $V_m$, with increasing pre-loading of the adsorbent with water vapour, expressed as a function of relative humidity. $V_m$ (solvent) + $V$ (water) = 2.0 ml (per gram of dry sorbent) = constant; *i.e.*, the higher the relative humidity to which the layer is exposed, the less solvent can be taken up by it and the smaller is the mobile phase (= dead volume). Kieselgel 60 (Merck) for TLC; layer thickness 1 mm; solvent, undecane; N-chamber with humidity control.

manually prepared plates), in CC it depends fairly strongly on the column packing density. The looser the packing, the greater is the dead volume.

In adsorption chromatography (LSC), moreover, the phase ratio is affected by the activity (*i.e.*, the water content) of the adsorbent. With an increasing amount of water, the dead volume decreases, as the free layer volume is reduced by the adsorbed water molecules. The remaining dead volume filled with solvent becomes the mobile phase, $V_m$. This is illustrated in Fig. 2.

The quantity of adsorbed water that reduces $V_m$ is added to $W_a$, thus enhancing the increase of the ratio $W_a/V_m$. Measured values for the phase ratio of Kieselgel 60 (Merck) are given in Table II. The parallelism of water content and $W_a/V_m$ is evident for TLC; for CC it is somewhat masked by variations of the packing density for the columns used.

## ADJUSTMENT OF THE ACTIVITY OF A COLUMN

A prerequisite for a successful transfer from TLC to CC is identical activity in both systems. The water content of the column adsorbent can be controlled by the

TABLE II

PHASE RATIO AS A FUNCTION OF THE WATER CONTENT OF AN ADSORBENT (KIESELGEL 60, MERCK)

| *Relative humidity (%)* | *Water in the adsorbent (%)* | $W_a/V_m$ | |
|---|---|---|---|
| | | *TLC* | *CC* |
| 23 | 5.6 | 0.54 | 0.71 |
| 38 | 9.4 | 0.55 | 0.65 |
| 51 | 13.2 | 0.62 | 0.65 |
| 67 | 23.0 | 0.71 | 0.75 |
| 77 | 38.0 | 0.84 | 0.97 |

following procedures: (1) spreading the column adsorbent side-by-side with the TLC plate, exposed to the atmosphere; (2) adding known quantities of water to the dry adsorbent; and (3) equilibrating the column with the appropriate and known water content of the solvent.

*Spreading of adsorbent in the air*

The easiest way to obtain equal activity for the layer and column is to spread the column adsorbent side-by-side with the plate in the ambient air[8]. This ensures equal but not necessarily optimum activity, as it depends on the particular relative humidity of the atmosphere, unless the ambient humidity is controlled adequately, *e.g.*, in a glove-box. Because of the "human factor", only in special cases does variation of the air conditioning of the whole laboratory seem feasible.

*Addition of water to the column adsorbent*

This is the classical way of adjusting Brockmann activities. To relate water contents to relative humidities (TLC), the water adsorption isotherms of the adsorbent must be known (*e.g.*, Fig. 3). The inconvenience of adding water to the powder arises because of non-homogeneous distribution and the risk of changing the activity during the filling procedure.

*Equilibration through the solvent*

The water content of the adsorbent in a column changes with time when the

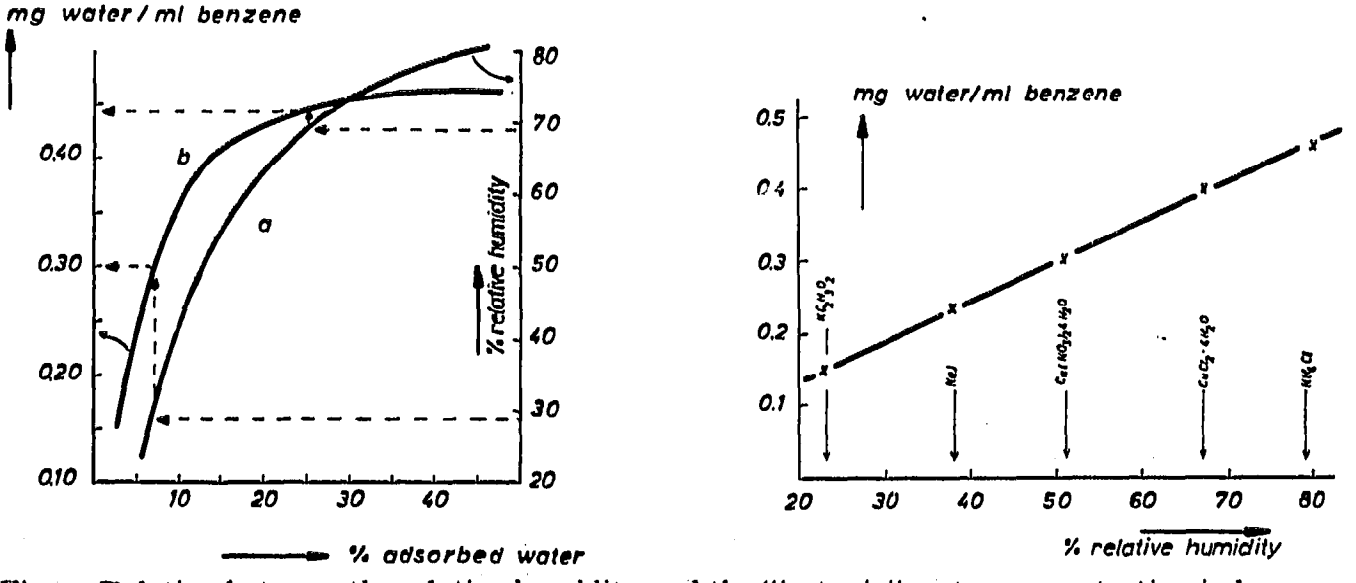



Fig. 3. Relation between the relative humidity and the "isotonic" water concentration in benzene with the equilibrium water content of Kieselgel 60 (Merck), 23 ± 2°. (a) Isotherm of water vapour adsorption (for the TLC activity). (b) Isotherm of liquid water adsorption in equilibrium with the mobile phase (for CC). Practical example: we require the "isotonic water content" of benzene for the CC which gives the same sorbent water content (*i.e.*, the same activity) that was present in the thin layer at 29% relative humidity. Following the arrows: 29% relative humidity in TLC corresponds to 7% sorbent water (curve a). A solvent water concentration of 0.30 mg/ml (= "isotonic water content") leads to the same adsorbed water concentration, 7%. This diagram is valid only for Kieselgel 60–benzene.

Fig. 4. Preparation of water–benzene mixtures using saturated solutions of different salts. Values of relative humidities and salt solutions taken from ref. 5.

solvent does not have the correct equilibrium water content, *i.e.*, the so-called isotonic water content[9]. When the solvent contains less water than that required for equilibrium, during operation the column is slowly dried and *vice versa*. These activity shifts become noticeable, for example, after the elution of one or two samples, *i.e.*, after the elution of 10 or 20 times the dead volume of the column. Thus, if an activity-adjusted column is used only once, its activity is not yet affected by the arbitrary water content of the solvent, nor is a thin-layer plate affected during a single run.

To make a virtue of necessity, one can also obtain the desired water content of the sorbent by adjusting the water content of the solvent appropriately. It is necessary to know the relationships between the relative humidity (for the TLC activity), the percentage of water in the column adsorbent and the isotonic concentration of water in the solvent. Such a set of conditions is valid for one adsorbent and one solvent only. An example for the couple Kieselgel 60/benzene is given in Fig. 3 (for operational description see the experimental section).

The desired water content can be obtained either by mixing known quantities of absolutely dry solvent and water-saturated solvents or by shaking a solvent of any water content with saturated solutions of inorganic salts (with deposit). For the latter case, Fig. 4 shows which salts give a particular water content in the solvent. An explanation is given in detail later of how such a transfer is carried out in practice.

## OTHER INFLUENCING FACTORS

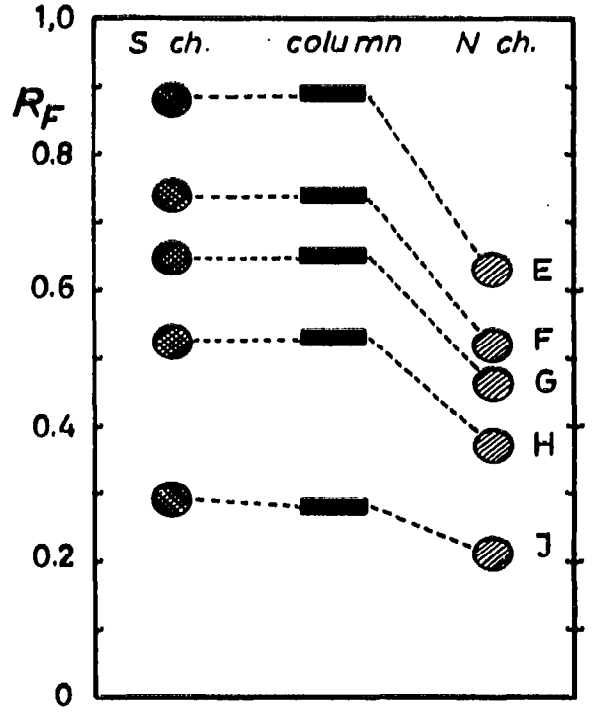
As has already been shown, adsorbents used for TLC and CC should be identical. They can differ only in grain diameter. There are now only very few commercially available sorbents that meet this condition. Pre-coated thin-layer plates may, because of their binder content, give other selectivities than the same column adsorbent without a binder. Also, most TLC sorbents can be used as column-filling materials, providing that they do not contain a binder.

Variations in the column temperature should not exceed $\pm 1°$, because, in contrast to TLC[10], CC fully reflects the influence of temperature. An increase in temperature reduces retention volumes. A temperature change can affect the resolution indirectly because it may change the selectivity and shift $V_s/V_m = k$ away from 2, its optimum value[11].

## WHAT IS TRANSFERABLE TO WHAT?

Before the development of the high-pressure variant of liquid column chromatography, it had been recommended to use "dry columns" for improved separations, *i.e.*, columns to which the sample is applied before the solvent. There is no essential difference in the bed quality when dry or wet columns are used. Also, the dry column is transformed automatically into a wet column if it is used for more than one sample, or in continuous operation. If a dry column is still used, when considering the transfer from TLC to CC the following points should be noted.

For both single and multicomponent solvents, chromatography in dry columns corresponds essentially to TLC development in an "unsaturated S-chamber"[12]. Solvent mixtures are separated and form multiple fronts. Practical examples for the transfer from TLC to a dry column for a single-component solvent are shown in



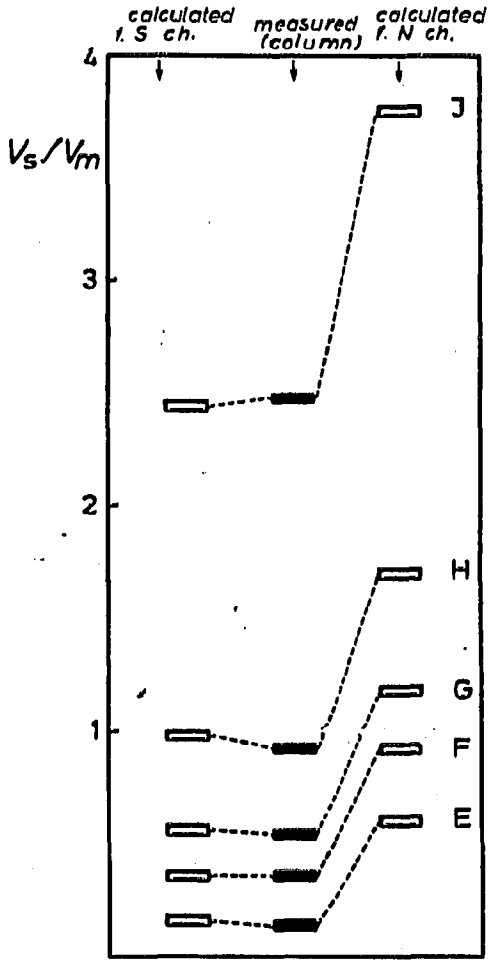




Fig. 5. Transfer from TLC to CC. Benzene, alumina T (Merck), 55% relative humidity. E = Butter yellow; F = impurity; G = sudan red; H = indophenol; I = sudan black. The $R_F$ values of the column were calculated from $V_s/V_m$ values by using eqn. 8.

Fig. 6. Transfer from TLC to CC. For data, see Fig. 5. $V_s/V_m$ values were calculated by using eqn. 8. Values at the left- and right-hand sides of the equation were calculated from S and N-chambers, respectively.

Figs. 5 and 6. Although the $V_s/V_m$ values were calculated only from the approximate relationship in eqn. 8, the agreement with the experimental values is good. Calculated $V_s/V_m$ values are much too high when $R_F$ values (with single-component solvents) were obtained from an N-chamber[12] (Figs. 5 and 6). To obtain the true $V_s/V_m$ value, the observed $R_F$ values must be multiplied by a constant factor $\xi$ (ref. 5). *Using a single-component solvent, there is no difference in the selectivities, when S- or N-chambers or dry or wet columns are used.*

For *multicomponent solvents*, the situation is more complex. A mixture of dyes that was developed with chloroform/ethanol in different chambers (Fig. 7) can be considered. In the S-chamber, component A migrates with $R_F \approx 1$ close to the front and clearly separated from B. Because of the pre-loading of the layer with solvent vapour in the N-chamber, the selectivity towards components A and B is changed in such a way that they are no longer separated (the upper part of the layer contains only chloroform in the S-chamber but chloroform + ethanol in the N-chamber).

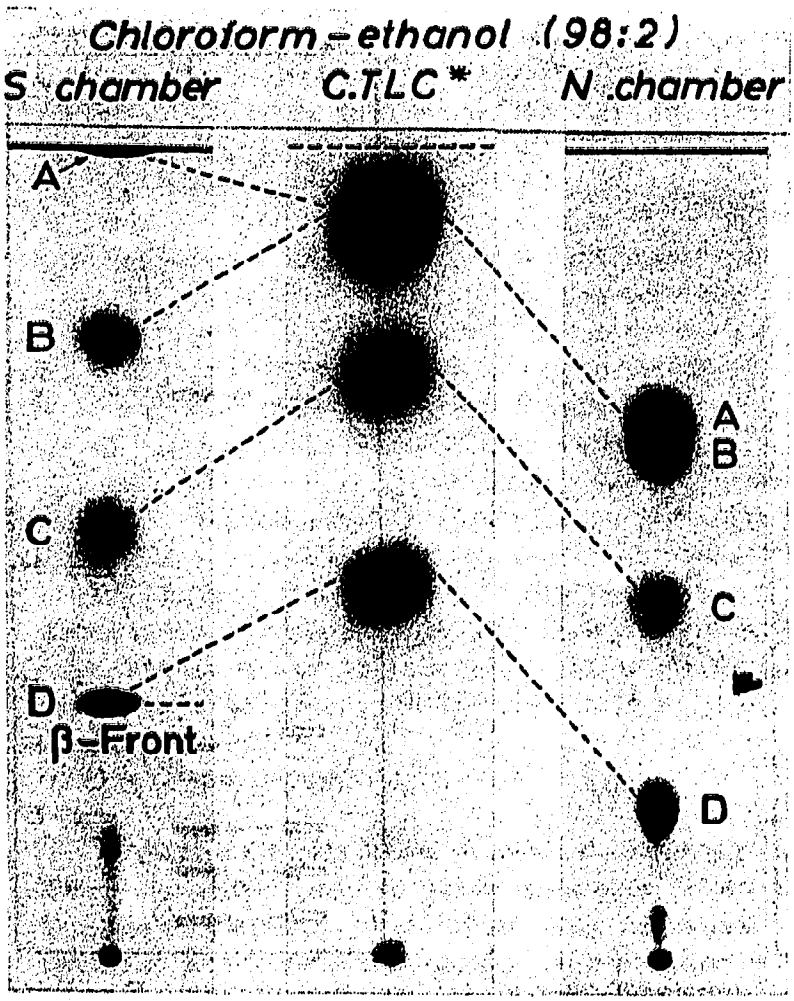



Fig. 7. Transfer from TLC to CC. Influence of chamber type using multi-component solvents. Alumina T (Merck), 55% relative humidity. A = Fat red; B = indophenol; C = acetorange; D = F8 (Ciba). For continuous TLC, the sample mixture was applied to the plate after the passage of the $\beta$-front using a BN-chamber.* Continuous TLC.

It might be thought that A + B (in Fig. 7) would migrate at the front, but because of the pre-loading they are lower (see ref. 5, p. 179) and migrate with the "imaginary front", which has an "$R_F$" value of 0.67, equal to $1/\xi = 1/1.5 = 0.67$. After correction they become $R_F' = 1$, hence $R_F' = (R_F)_{obs.} \cdot \xi = 0.67 \cdot 1.5 = 1$.

Component D is migrating in the S-chamber together with the $\beta$-front, which is perceptible at the spot compression. The chromatogram in the middle of Fig. 7 was obtained in a horizontal BN-chamber with a continuous flow of solvent mixture. The sample was applied to the wet plate after the $\beta$-front had passed the starting line. Because of the addition of the dissolved sample to a liquid, instead of to a dry plate, the spot became more diffuse. This chromatogram, apart from $R_F$ differences, resembles that of the N-chamber. In Fig. 8 the $R_F$ values of the three chromatograms shown in Fig. 7 with the $R_F$ values calculated from eqn. 8 are compared by using $V_s/V_m$ values for this sample obtained with the same solvent in a dry and a wet column, respectively. The following conclusions can be drawn (for the solvent mixture): (1) the results in the S-chamber and dry column are identical, as expected;

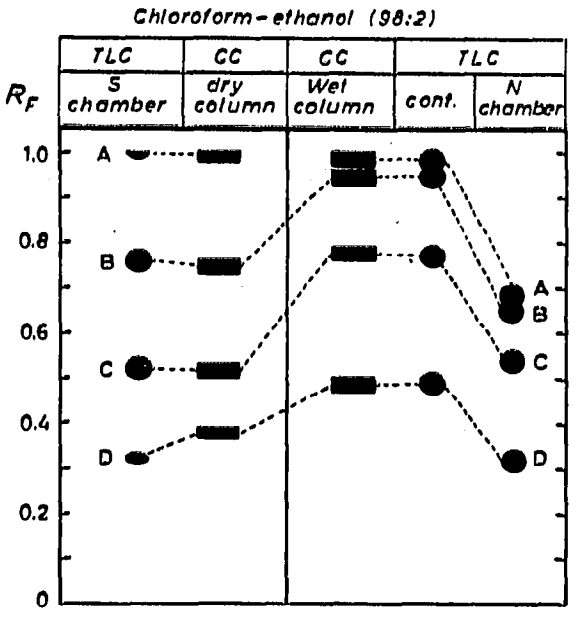



Fig. 8. Transfer from TLC to CC. Solvent mixtures. $R_F$ values calculated from the separations in Fig. 7, compared with data obtained from separations in dry and wet columns.

(2) the results of the S-chamber cannot necessarily be transferred to the wet column; (3) the corresponding technique for the wet column is, to a certain approximation, N-chamber development (see later) and, more precisely, continuous TLC in an S-type chamber; (4) the separation in the N-chamber does not correspond to the results in the dry column; in the N-chamber ethanol is present over the entire length of the layer, whereas in the dry column A, B and C always migrate in an ethanol-free zone.

These observations are summarized in Table III.

*Details of the transfer from N-chamber to wet column for solvent mixtures*

Continuous TLC is not a rapid technique and therefore will not be used by many people as a pilot technique for CC. The combination N-chamber–wet continuous column will probably be used, as N-chambers, although not ideal, are the most widespread in use.

When a plate is developed in the usual way in an N-chamber with a solvent mixture of components of widely varying strengths, at the end of the development there will be a concentration gradient of these components along the layer (ref. 5, Fig. 57) whose shape and steepness depend on the relative strengths, vapour

TABLE III

TRANSFERABILITY OF TLC RESULTS TO A COLUMN

| *Pure solvents* | | | *Solvent mixtures* |
|---|---|---|---|
| S-chamber | → | dry or wet column | S-chamber → dry column<br>S-chamber ⫽→ wet column |
| N-chamber · ξ | → | dry or wet column | N-chamber · ξ → wet column<br>N-chamber ⫽→ dry column<br>Continuous TLC → wet column |

⫽→ = not transferable.

pressures and concentrations of the solvent components, as well as on the dimensions of the chamber and the nature of the layer. This concentration gradient necessarily causes a certain selectivity gradient along the layer, which, in a column with its stationary operation at equilibrium, is absent. To make the concentrations on the plate more uniform, it is better to expose the layer in the N-chamber to the solvent vapour at chamber saturation[12] before starting the development. Then the probability of having homogeneous conditions for about 20 min during and at the end of development becomes greater. The same effect can easily be achieved with a KS-chamber.

There is another uncertainty inherent in the use of solvent mixtures. Until now there have been no reliable rules on how the water content in the layer can and must be controlled in the ternary system water–solvent A–solvent B or in more complex systems. Isotonic water contents for solvent mixtures are not known. Fortunately, the stronger the solvent, the less effective becomes the water content of the layer. In this respect, the tolerances have not yet been investigated.

When solvent mixtures are used in columns, injection of the sample cannot be carried out before the strongest solvent component has "broken through" the column; only then is the column in stationary equilibrium with all the solvent components. This may take a long time (hours or days), especially when the strong component is present only in very small concentrations. This difficulty can be overcome by wetting the dry column first with the pure, strong component, and adding the final solvent mixture only after the breakthrough of the initial solvent. Thus the equilibration process (to be controlled by a constant detector response) is essentially accelerated.

## PROOF OF THE VALIDITY OF THE TRANSFER FORMULAE

In addition to the practical example for a transfer described earlier, another example will be given of the validity of eqns. 5 and 9, especially with respect to the

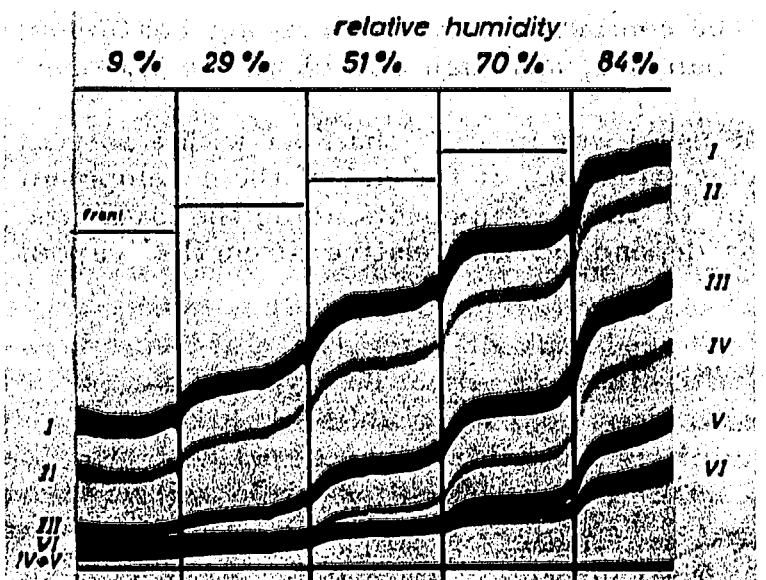



Fig. 9. TLC separation of dyes at different relative humidities. Kieselgel 60 (Merck); KS-chamber; benzene. I = Butter yellow; II = impurity; III = sudan red; IV = indophenol; V = sudan black; VI = *p*-hydroxyazobenzene.

precise calculability of the factor $k_f$, even along a controlled activity shift. The prerequisites for this experiment were well defined conditions with a single-component solvent.

The separation of a dye mixture containing butter yellow (I), impurity(II), sudan red (III), indophenol (IV), sudan black (V) and *p*-hydroxyazobenzene (VI) by TLC with Kieselgel 60 (Merck) and benzene was to be transferred to a column filled with the same adsorbent. A complete separation of all components can be achieved only at a relative humidity greater than 51%, as the results of an orthogonal[2,13] TLC activity gradient (Fig. 9) show.

It was then decided first to bring the column to an activity corresponding to a relative humidity of 69%, *i.e.*, 25% water in the adsorbent (from curve a in Fig 3) by equilibrating with the isotonic solvent benzene containing 0.44 mg of water per

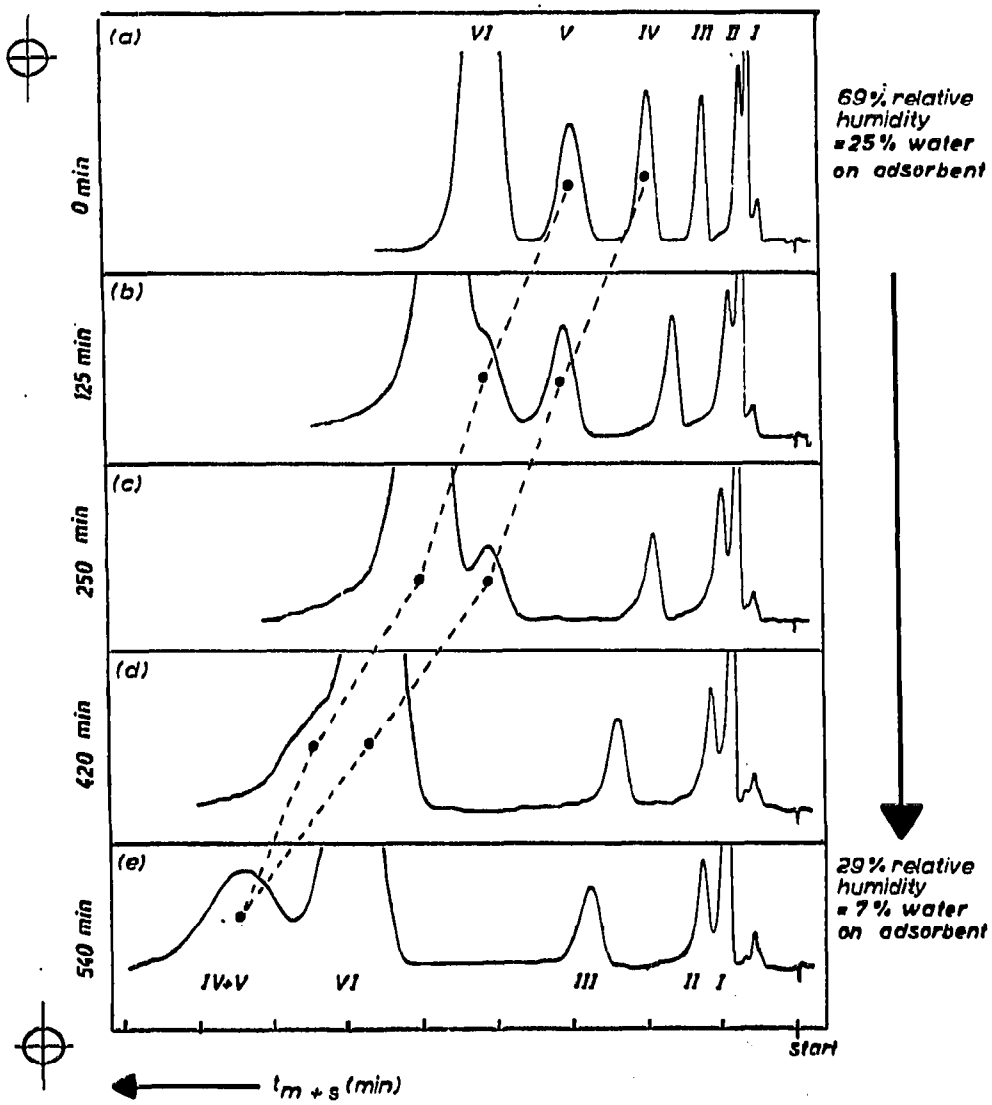



Fig. 10. Consequences of an activity shift in the column after substituting a wet solvent (0.44 mg of water per ml of benzene) by a drier solvent (0.30 mg of water per ml of benzene). (a) Wet solvent in equilibrium. Solvent was changed after this chromatogram. (b) to (d) Chromatograms indicating the slowly changing intermediate activity states. At (e), after 540 min, the new equilibrium is reached. Compounds IV, V and VI have changed places, *i.e.*, the change of activity involves change in selectivity.

ml (from curve b in Fig. 3) and then to replace this solvent with benzene with 0.30 mg of water per ml (corresponding to a relative humidity of 29% and 7% of water in the adsorbent), and to follow the evolution of the chromatograms resulting from this change in the water content of the solvent (Fig. 10). The upper chromatogram (a) shows the results under the starting conditions (relative humidity 69%). The order of elution is, as in TLC, I, II, III, IV, V, VI. Under the permanent drying effect of the new solvent, the chromatogram is continuously changing and through the photographs (b), (c) and (d) is moving progressively to the equilibrium state at (e), which is reached after 650 ml of solvent have passed the column (the time of "trans-activation" is 9 h at a flow rate of 1.20 ml/min.).

It can be seen that: (1) the absolute retention times increase; (2) the substances IV and V are no longer separated; (3) their elution order with VI is inverted; and (4) the resolution of I and II is increased.

Figs. 11a and b show the comparison of $V_s/V_m$ values measured in the column and calculated from eqn. 5 from $R_F'$ values, for the two extreme activities at 69% and 29% relative humidity. The following phase ratios were used:

$(W_a/V_m)_{CC}$ = 0.92 at 69% relative humidity
$(W_a/V_m)_{TLC}$ = 0.71 at 69% relative humidity
$(W_a/V_m)_{CC}$ = 0.71 at 29% relative humidity
$(W_a/V_m)_{TLC}$ = 0.55 at 29% relative humidity

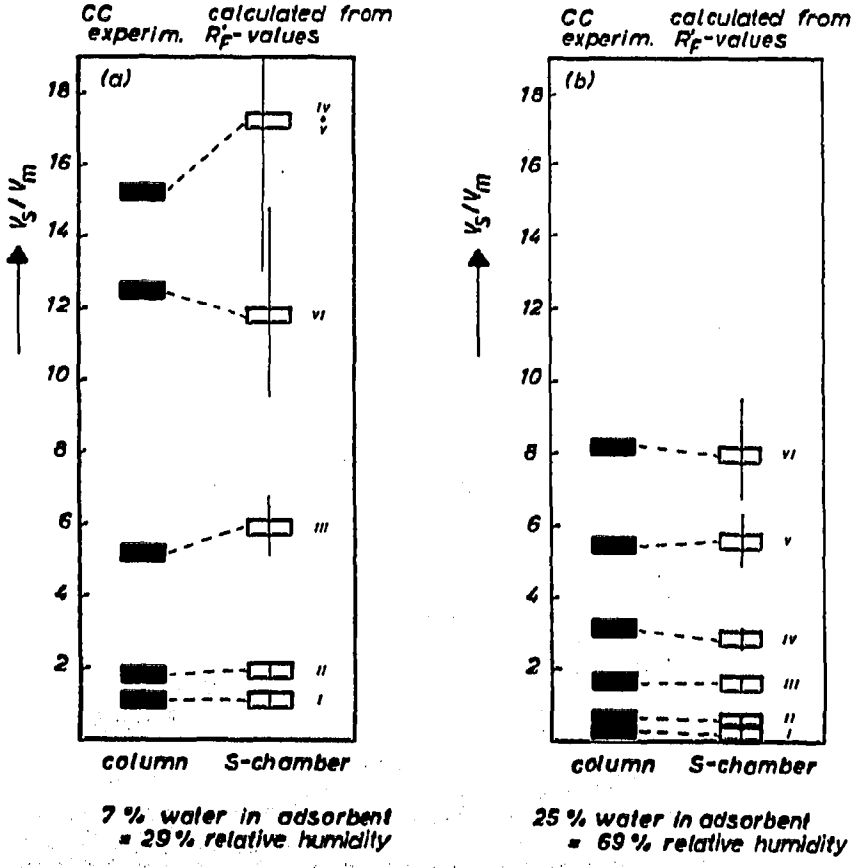



Fig. 11. Comparison of data calculated from TLC $R_F$ values (Fig. 9) with $V_s/V_m$ values measured by CC (Fig. 10) at the same sorbent water content (eqn. 5). The vertical lines in the calculated values correspond to the TLC reproducibility range of $\pm 0.02$ $R_F$ units.

An example of the calculation is given below for sudan red (III), $R_F' = 0.44$ at 69% relative humidity.

$$V_s/V_m = (0.92/0.71) \cdot [(1/0.44) - 1] = 1.30 \cdot 1.28 = 1.67$$

The experimental value measured using the column (after equilibration with benzene containing 0.44 mg of water per ml) was 1.71, which is in excellent agreement with the calculated value.

## CONCLUSIONS

This paper is intended to show how TLC separations can be transferred precisely and reliably to columns when the working conditions are well defined. The governing factor for lipophilic single-component solvents is the activity. Admittedly, in most instances an over-precise prediction of retention volumes from $R_F$ values will be only of academic interest provided that the selectivity and separations can be transferred. However, this possibility should not be left to accident. A rapid check on TLC with an orthogonal gradient (*e.g.*, in a KS-chamber) can demonstrate whether small changes in activity or solvent composition will seriously affect the results or not.

For solvent mixtures, the margin of potential error in the transfer is greater than for single-component solvents (an investigation of this, especially with respect to the influence of water as the ternary component, has not yet been carried out). However, the basic validity of the transfer formulae (eqns. 5 and 9) is not affected by these experimental uncertainties. On the other hand, recent results[5,14] have shown that, for the chromatography of certain types of compounds, the selectivity can be increased using "equielutropic"[5,11,15] solvent mixtures. Optimizing the selectivity by changing columns and solvent composition is rather cumbersome. It is better to carry out pilot tests on TLC, especially with orthogonal vapour pre-loading gradients[2,5], occasionally accompanied by a simultaneous multi-solvent development, both carried out in a KS-chamber. Thus the claim that TLC is a valid and efficient pilot technique for CC, both for single and multi-component solvents, is justified. This is obviously not true when CC sorbents are not available in TLC quality, which is the case for almost all of the new solid-core porous-layer adsorbents coated or not with "bonded" organic phases.

This paper has not dealt with problems of optimizing the bed quality of the column or other instrumental features of the liquid chromatograph.

## EXPERIMENTAL

The N-chamber[5] is the conventional ("normal") TLC trough chamber whose walls are lined with filter-paper. An unsaturated S-chamber is a sandwich chamber without a wetted counterplate. The separations with controlled activity were carried out in the Vario-KS-chamber[2,5] (Camag, Muttenz, Switzerland).

All the column separations corresponding to Figs. 5–8 were carried out in a 30-cm glass tube of 10 mm I.D., with a dropping funnel and hydrostatic pressure and with UV detection. TLC plates and CC adsorbents were exposed to the atmosphere (at 55% relative humidity) before use. The effluent water content was not controlled.

The separations in Figs. 1, 10 and 11 were carried out with the Universal-

Flüssigkeitschromatograph UFC-1000 (Hupe & Busch, Karlsruhe-Grötzingen, G.F.R.). The operating conditions comprised 50-cm glass capillaries of 2 mm I.D., 120 atm pressure and flow-rate 1 ml/min. The temperature of the column and empty pre-column was controlled thermostatically at 20°. The columns were filled with the dry adsorbent by striking, by using the method of LOEV and co-workers[16,17].

The phase ratio in TLC was determined in the following way. A glass plate, 3 × 10 cm, was weighed before and after coating and climatization (the difference = $W_a$). After development with "overrun"[18] with undecane, the weight of the liquid in the layer was determined; $V_m$ was obtained after division by the density. To determine the phase ratio in CC, $W_a$ was determined by weighing the column before and after filling with the climatized sorbent. $V_m$ was determined from the difference in weight between the dry and the wet column. $V_m$ can also be determined by injection of a compound with $R_F \approx 1$; then $V_m = V_{m+s}$. The water content of the adsorbent in the isotherm of Fig. 3 is referred to a dry weight at 125°.

The "liquid isotherm" in Fig. 3 relates the equilibrium water content of the adsorbent to the water content of the solvent. It was determined in the following way. A column with a known water content (greater than the equilibrium content) was flushed with a drying solvent of known water content until the water content of the column effluent had decreased to the water concentration of the entering solvent. The eluted excess of water was titrated by the Karl Fischer method and subtracted from the initial water content of the adsorbent. The remainder of the water is equal to the equilibrium water content of the adsorbent in contact with the solvent. This is the highest point of the isotherm. The other points are obtained by stepwise reduction of the solvent water concentration, and analogous titrations.

The different water contents of the solvents were adjusted by vigorously shaking them with different saturated solutions of inorganic salts in water (see Fig. 4) for 3 h at 20 ± 1°.

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