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THIN-LAYER CHROMATOGRAPHY AS A PILOT TECHNIQUE FOR RAPID COLUMN CHROMATOGRAPHY (SUMMARY)*

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INTRODUCTION

Since both thin-layer chromatography (TLC) and column chromatography (CC) are forms of liquid chromatography (LC), 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 a plate[1], which has an available number of separation plates limited by its maximum length (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 LC 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. 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; in TLC there is no compound "irreversibly retained" as 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 can be complementary: TLC is then used to pre-optimize the separation conditions.

FORMULAE FOR TRANSFER FROM TLC TO CC

For a given set of conditions, *i.e.* adsorbent, solvent, activity, and $0.2 < R_F < 0.8$, the following transfer formula is valid:

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

where

V_s = net retention volume of the column
V_m = dead volume
k_f = constant = $(W_a/V_m)_{CC}/(W_a/V_m)_{TLC}$
W_a = weight of adsorbent in layer or column
V_s/V_m = k', "capacity factor" or "partition number".

The constant k_f can be determined by chromatographing any compound by both techniques. For any other compound, V_s/V_m can then be calculated from $(R_F)_{TLC}$.

ADJUSTMENT OF THE ADSORBENT ACTIVITY

A pre-requisite for a successful transfer from TLC to CC often is identical activity in both systems. First, the water content in the layer is optimized using a Vario KS chamber. Then the corresponding activity of the column is obtained either by addition of the appropriate amount of water to the dry adsorbent or by equilibration through the "isotonic" solvent, with equilibrium water content.

TYPE OF DEVELOPMENT TANK AND TRANSFER

(1) In paper-lined "N-tanks" the dry layer is pre-loaded ("pre-equilibrated") by solvent vapours. This pre-loading is prevented in sandwich-type tanks ("S-tanks").

(2) Comparison is only possible between TLC results obtained in N-tanks and those obtained in "wet" columns, especially when solvent mixtures are used. A transfer of TLC data obtained in S-tanks with solvent mixtures to those from wet columns will lead to unsatisfying results.

(3) For theoretical reasons, R_F values can never reach 1.00 in developing tanks of the N-type (with vapour pre-loading), their maximum is *ca.* 0.8.

(4) Also for theoretical reasons, resolution on a thin-layer plate tends necessarily towards zero for $R_F \to 1.0$ in an S-tank and for $R_F \to 0.7$ or 0.8 in an N-tank. This must be kept in mind when analysing "insufficient" at very high R_F values. It is possible to avoid this by changing the experimental conditions in such a way as to lower the R_F values. Maximum resolution is by $R_F \sim 0.3$.

(5) An essential increase of selectivity is often obtained by using mixtures of a very small portion of strong solvent with an excess of weak solvent.

REFERENCES

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