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Note

A graphical method for the determination of retention times in column liquid–solid chromatography from thin-layer chromatographic data

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The advantages of thin-layer chromatography (TLC), stressed by a number of workers (*e.g.*, refs. 1–4), have frequently been utilized to estimate retention behaviour and to choose and optimize solvent systems in column chromatography[4–11].

Early investigators did not take into account fundamental differences between TLC and column chromatography and thus were limited to approximate, qualitative characteristics of the solvent–adsorbent system. More recently, Schlitt and Geiss[3,4] derived equations that permit the use of TLC as a pilot technique for column chromatography on a quantitative basis. Moreover, they also defined the experimental conditions that are necessary to secure analogy between TLC and column chromatography.

The equations derived by Schlitt and Geiss[3] require the determination of certain auxiliary parameters and the calculations are time consuming. Therefore, in this work we have attempted to simplify the procedure by the application of a graphical correlation method, using principles employed earlier in a method for the determination of suitable solvent systems for column liquid–liquid partition chromatography or countercurrent distribution from paper chromatographic data[12] and in a comparison of TLC parameters in two different experiments[13]. The graphical correlation method permits the comparison of large sets of experimental data (see also a recent paper by Coq *et al.*[14] in which graphical correlation in another coordinate system is employed).

In order to secure linear relationships and a wide range of k' values (k' = amount of solute adsorbed/amount of solute in the mobile phase), it is advantageous to use a logarithmic scale for capacity factors. Therefore, the R_M value is used as the TLC parameter:

$$R_M = \log \frac{1 - R_F}{R_F} = \log k'_{(\mathrm{TLC})} = \log k^\circ + \log \frac{W_{A(\mathrm{TLC})}}{V_{S(\mathrm{TLC})}} \qquad (1)$$

For column chromatography (CC), the logarithm of the capacity factor is calculated from the equation

$$\log k'_{(\mathrm{CC})} = \log \frac{t_R - t_R^\circ}{t_R^\circ} = \log k^\circ + \log \frac{W_{A\,(\mathrm{CC})}}{V_{S\,(\mathrm{CC})}} \qquad (2)$$

It follows from eqns. 1 and 2 that for identical solvent systems used in the two techniques (the same developing solvent, the same adsorbent of identical activity), the k° values are identical and the difference in k' values in TLC and column chromatography results from differences in the ratios of the weight of the adsorbent and the volume of the solvent, W_A/V_S. If the ratio is identical in both methods and the migration rates of solutes are described by eqns. 1 and 2, then $R_M = \log k'_{(CC)}$ and the correlation line of $\log k_{(CC)}$ *versus* R_M passes through the origin, the slope being 1.0. In the opposite case, when the W_A/V_S ratios are different for the two methods, then the straight correlation line is shifted (vertically and horizontally) in the logarithmic scale by

$$\log \left(\frac{W_{A\,(TLC)}}{V_{S\,(TLC)}} \bigg/ \frac{W_{A\,(CC)}}{V_{S\,(CC)}} \right)$$

To test the graphical method, several organic nitrogen bases were chromatographed using thin layers and columns of silica gel.

EXPERIMENTAL

Column chromatography was carried out using a DuPont Model 830 liquid chromatograph with a UV detector. A steel column, 50 cm × 2.1 mm I.D., was packed with wide-pore silica (average pore diameter 120 Å, particle size 10–50 μm, produced by Polish Reagents, Gliwice, Poland). Small portions of silica (dried at 140° for 2 h) were introduced into the tube, the column being tapped 50 times against a wooden bench between each addition. Reagent-grade solvents dried over molecular sieve 13X were used. The column was developed with binary solvent mixtures; solutions of single solutes (3–10 μl of 2.5%, w/v) were injected after equilibration of the column. The experiments were carried out at 25 ± 1°, the flow-rate being kept constant at 0.7 ml/min.

Ascending TLC was carried out using 10 × 20 × 30-cm glass tanks lined with filter-paper. Glass plates, 18 × 20 cm, were covered with 0.25-mm layers of the same silica used in the column experiments. The samples (solutions in acetone or methanol) were spotted 2 cm from the edge and dried with a stream of warm air so as to prevent adsorption of water vapour. The solvents, prepared as in column chromatography, were poured into the tanks and after conditioning in the vapour (30–40 min) the plates were developed to a distance of 16 cm. The spots were revealed with Dragendorff reagent ($KBiI_4$) or bisdiazotized benzidine.

RESULTS AND DISCUSSION

The results are presented in Figs. 1–3. For practical reasons, double scales are marked on the two coordinates: on the R_M axis a subordinate scale of R_F values[15,16] (which can be found directly from the thin-layer chromatograms) and the relative retention time t_R/t_R° on the $\log k'$ axis. The plot thus allows one to estimate relative retention times (or relative retention volumes, V_R/V_R°) directly from experimentally determined R_F values.

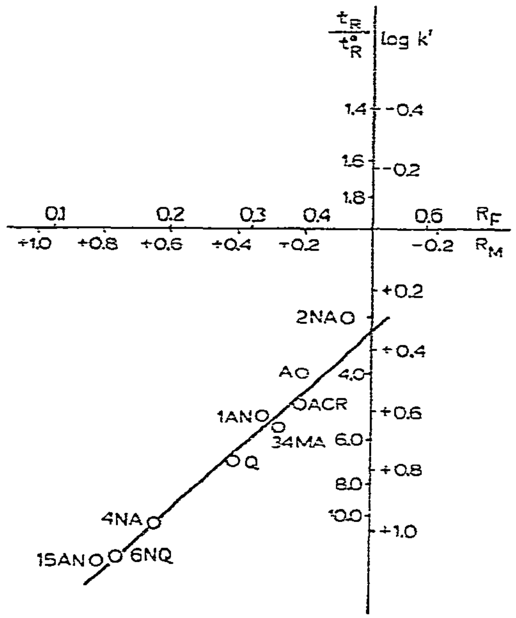

Fig. 1. Correlation of logarithms of capacity ratios obtained by TLC (abscissa) and column chromatography (ordinate). System: silica–ethyl acetate (20 mol-%) + cyclohexane. Solutes (Figs. 1–3): aniline (A) and its 2-nitro-(2NA), 4-nitro- (4NA) and 3,4-dimethyl- (34MA) derivatives; 1-amino- (1AN) and 1,5-diaminonaphthalene (15AN); 1,2-diaminobenzene (12AB); quinoline (Q); 6-nitroquinoline (6NQ); α-naphthoquinoline (αNQ); and acridine (ACR).

The equations of the correlation lines determined by the least-squares method are:

Fig. 1 (20 mole-% ethyl acetate): $\log k'_{(CC)} = 0.98\ R_M + 0.34$
Fig. 2 (10 mole-% ethyl methyl ketone): $\log k'_{(CC)} = 1.10\ R_M + 0.17$
Fig. 2 (60 mole-% ethyl methyl ketone): $\log k'_{(CC)} = 1.07\ R_M + 0.25$
Fig. 2 (both compositions): $\log k'_{(CC)} = 0.99\ R_M + 0.19$
Fig. 3 (10 and 40 mole-% ethyl acetate): $\log k'_{(CC)} = 1.01\ R_M + 0.22$

The slopes of the correlation lines are thus close to unity, in accordance with theoretical expectation. The lines are shifted from the origin by *ca.* 0.2–0.3 unit, which could have been due to higher ratios of adsorbent weight to solvent volume in column chromatography or other causes, such as differences in adsorbent activities in the two techniques, kinetic effects, demixing of the solvents and non-equilibrium conditions in TLC.

It is worth mentioning that linear correlations are observed even for different compositions of the developing solvent of a given type (Figs. 2 and 3) and even for different solvent systems (the last two correlation equations for Figs. 2 and 3 are virtually identical).

In the theoretical considerations, idealized chromatographic processes were assumed; in reality, the relationships can be distorted by additional effects, such as demixing of the solvents and non-equilibrium conditions in TLC. It has been demonstrated[17] that under comparable conditions (identical adsorbent, conditioning of

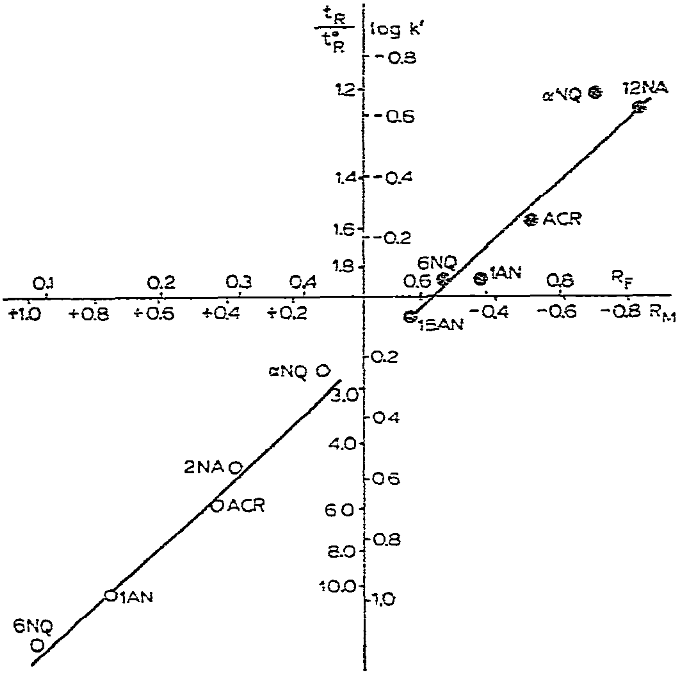

Fig. 2. As Fig. 1, with developing solvent ethyl methyl ketone (S) + cyclohexane. Open circles, 10 mole-% S; closed circles, 60 mole-% S.

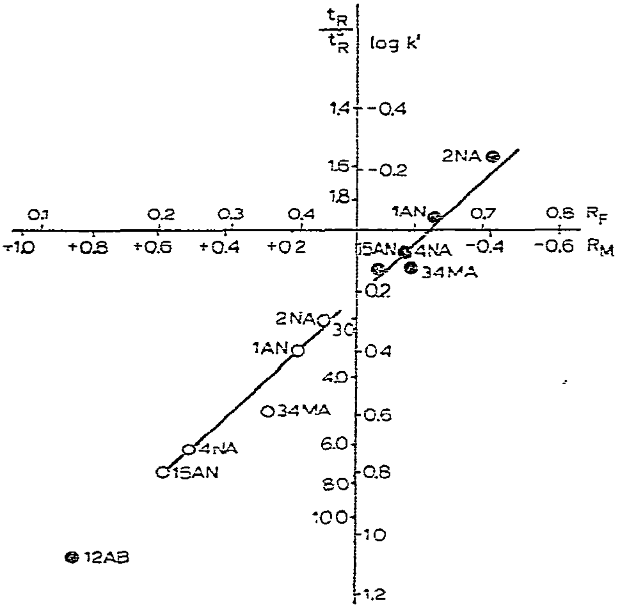

Fig. 3. As Fig. 1, with developing solvent ethyl acetate (S) + benzene. Open circles, 10 mole-% S; closed circles, 40 mole-% S.

the plates in TLC, use of sufficiently large tanks saturated with solvent vapour) the mechanisms of adsorption processes in TLC and column chromatography are identical or very similar.

For adsorbents of the same type differing in specific surface area ($a_{(TLC)}$, $a_{(CC)}$) the correlation line should be shifted by log ($a_{(TLC)}/a_{(CC)}$).

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