Journal of Chromatography, 168 (1979) 230–233

CHROM. 11,343

Note

Stepwise gradient development in sandwich tanks for quasi-column thin-layer chromatography

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(Received June 2nd, 1978)

The separation of samples containing components of widely different polarities is difficult owing to the "general elution problem"[1,2], which in column chromatography can be solved by the application of gradient elution. The technique can also be applied in thin-layer chromatography (TLC)[3,4], but the technical difficulties of obtaining reproducible gradients have so far limited the application of gradient TLC. It seems that the sandwich tank for quasi-column TLC can present new possibilities in the direction, especially for stepwise development[5]. The delivery of the solvent by a capillary siphon and rapid distribution at right-angles to the direction of development[6,7] permit the composition of the solvent to be changed in a simple manner by substitution of containers[8].

The choice of the development programme will first be illustrated by a hypothetical case. Let the R_M *versus* solvent composition plots of solutes A–G be as represented in Fig. 1. It is apparent that isocratic development cannot separate all of the components into single spots; *e.g.*, at a 10% concentration of the polar solvent the most polar solutes, E, F and G, remain on the starting line (Fig. 2a), while development with pure polar solvent cannot separate components A–D (Fig. 2b). The choice of an optimal two-stage elution gradient can be made on the basis of R_M *versus* log(% composition) plots (Fig. 1). If the first solvent will be used to develop the plate to $R_F = 0.5$ its composition is chosen so that about half of the solutes (A, B and C) have R_F values above 0.5. These solutes will then remain in the upper part of the developed area at all times. The second composition should secure a good separation of the remaining solutes (D–G). In Fig. 2c, the migration of the components during development with 10% solvent to $R_F = 0.5$ and further development with 100% polar solvent to the end of the plate is illustrated; it results in even spacing and complete separation of all components.

For sandwich development, solvent demixing effects can distort the theoretical picture owing to the formation of a steep gradient due to depletion of the polar solvent in the proximity of the solvent front[9]. However, as Wawrzynowicz and Soczewiński demonstrated[10], the R_F of the solvent demixing front is high for typical mixtures of non-polar and polar solvents except for concentrations of the polar component below 10% when the R_F values of the demixing front are lower than 0.8. To avoid overtaking of the front by sample spots (which would cause a decrease in selectivity), the sample should be spotted several centimetres behind the observed

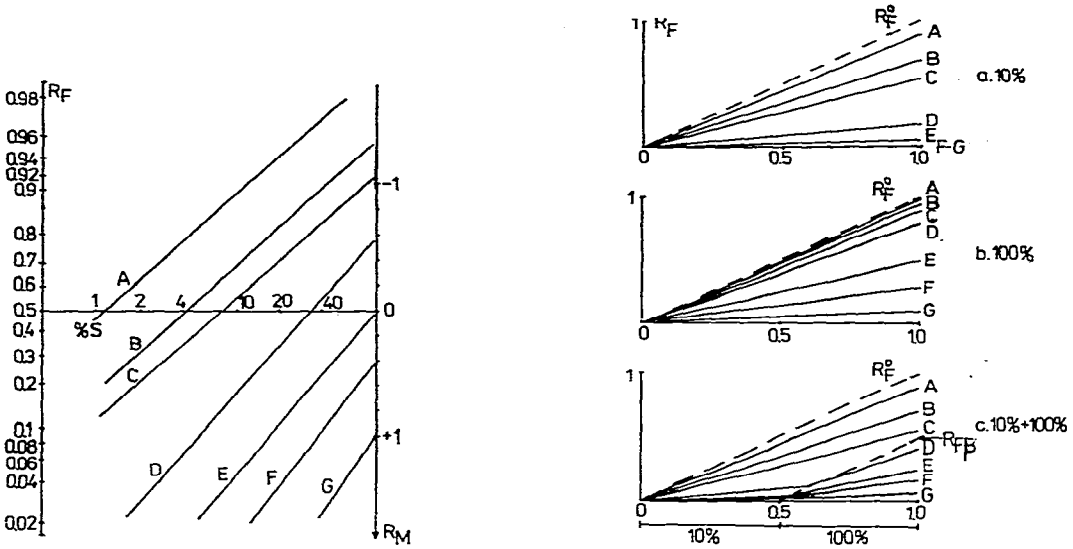

Fig. 1. R_M *versus* log (% S) plots for hypothetical solutes A–G with a subordinate R_F scale.

Fig. 2. Migration of solutes represented in Fig. 1 during isocratic (a,b) and stepwise development (c): (a) 10% solution of polar solvent (E,F and G not separated); (b) pure polar solvent (A, B, C and D not separated); (c) development with 10% polar solvent to $R_F = 0.5$ and continuation with 100% polar solvent (all solutes separated). Broken lines, migration of the non-retained marker solute (R_F^0) and of the front of increased concentration of the polar solvent ($R_{F\beta}$). R_F denotes the position on the plate

solvent front with a marker (indicator of the solvent flow-rate)[5–7] of $R_F = 1.0$. Analogous elimination of solvent demixing can also be achieved by pre-equilibration of the adsorbent layer in the vapours of the polar solvent[10]. To apply numerous samples in an even line on wider plates, the solvent flow can be stopped by disconnecting the distributor from the edge of the thin layer (Ref. 7, Fig. 1a). It should be emphasized that the sandwich tank development requires very small volumes of the solvent, *e.g.*, *ca.* 2 ml for 10 × 20 cm layers of thickness 0.3 mm.

EXPERIMENTAL

Glass plates (5 × 20 cm) were covered with 0.25-mm layers of silica gel after Stahl (SI 60; E. Merck, Darmstadt, G.F.R.), dried in air and activated for 1 h at 80° and 2 h at 130°. The solutes were spotted several centimeters behind the solvent front as 0.5% benzene solutions. Azobenzene was used as the marker of solvent flow-rate. The chromatograms were developed with 5, 10, 20, 30, 40, 50 and 60% (v/v) solutions of methyl ethyl ketone in cyclohexane; in stepwise elution 5% and 50% solutions were used. The chromatographed solutes were aromatic amines; N,N-dimethylaniline (A), N-ethylaniline (B), aniline (C), *o*-, *m*- and *p*-phenylenediamine (D, E and F, respectively) and 3-aminopyridine (G).

RESULTS AND DISCUSSION

The mixture of solutes is of the type discussed in the introduction. It follows from the experimental R_M *versus* log(% methyl ethyl ketone) plots (Fig. 3) that it can-

not be separated by isocratic development (Fig. 4). At a 5% concentration of methyl ethyl ketone solutes A and B can be separated and their R_F values are above 0.5; the remaining bases can be separated at higher concentrations of ketone in the mobile phase, *e.g.*, 50%. Therefore, stepwise gradient development was carried out in the following manner. To avoid solvent demixing effects, the mixture was spotted 4 cm behind the front of the 5% methyl ethyl ketone solvent together with azobenzene as a marker. When the azobenzene spot had migrated to the middle of the plate, the container of the solvent was replaced with another container of 50% methyl ethyl ketone and development was continued until the marker spot had migrated to the end of the plate. The chromatogram was then dried and the spots of the amines were detected by spraying with aqueous sodium hydrogen carbonate and then with bis-diazotized benzidine. The six components formed separate spots on the chromatogram (Fig. 4).

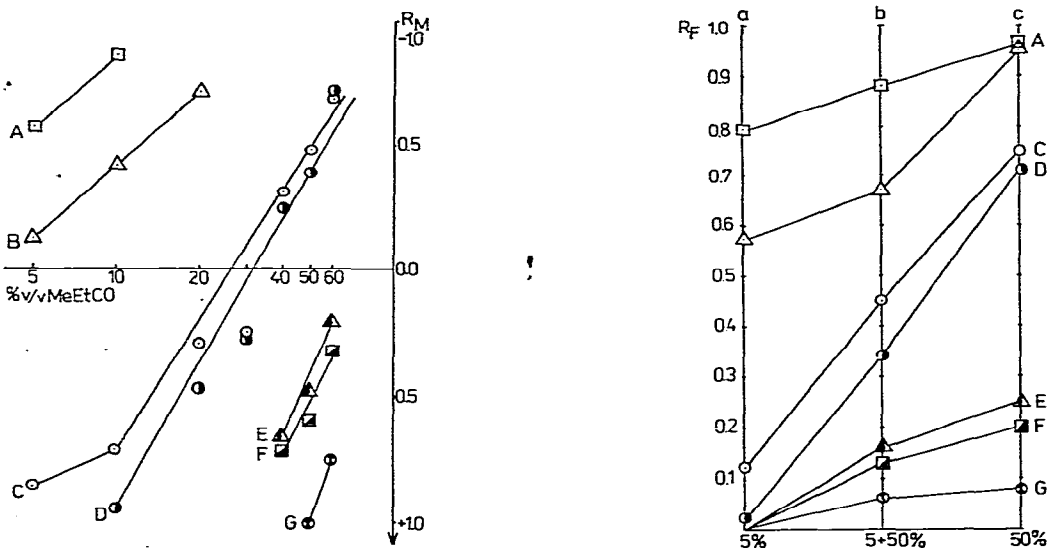

Fig. 3. Experimental R_M *versus* log(% methyl ethyl ketone) plots for aromatic amines. Diluent, cyclohexane.

Fig. 4. R_F values of aromatic amines. (a,c) Isocratic development with 5 and 50% solutions of methyl ethyl ketone, respectively; (b), stepwise development with both solvents.

It should be noted that for wider plates (*e.g.*, 10–20 cm) the change in solvent composition at the extremes of the distributor occurs with some delay so that a crescent-shaped front of increased concentration of ketone is produced. This effect can be eliminated by the following modification of development (Fig. 5).

(1) The capillary siphon of the first container is removed and the cover plates moved further along the thin layer to position (c), in which the remainder of the solvent is absorbed evenly by the layer.

(2) The cover plates are moved back to the initial position (a) and the space under the distributor is filled with the solvent from the second container.

(3) The distributor is again brought into contact with the layer [position (b)].

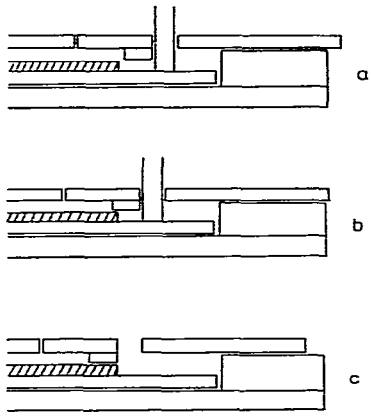

Fig. 5. Formation of even fronts of increasing concentration of polar solvent on wide plates. (a) Filling the space under the distributor; (b) development; (c) delivery of the solvent is stopped and the remainder of the solvent is absorbed by the layer.

It has been verified by using solvents coloured with non-polar dyes that this procedure (which can also be used to apply even bands of the sample to be separated) produces straight fronts of varying solvent concentration.

ACKNOWLEDGEMENT

These investigations were supported financially by the Polish Academy of Sciences (Problem No. 03.10).

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