Journal of Chromatography, 176 (1979) 247–254

CHROM. 11,882

Note

Use of equilibrium sandwich tanks with a glass distributor in band application and micropreparative separation of organic compounds

EDWARD SOCZEWIŃSKI and WIESŁAWA MACIEJEWICZ
Department of Inorganic and Analytical Chemistry, Medical Academy, 20-081 Lublin (Poland)
(First received December 12th, 1978; revised manuscript received March 20th, 1979)

It has been mentioned in an earlier paper[1] that the glass distributor for sandwich tanks[2–4] which permits the composition of the developing solvent to be changed rapidly[1,5] can also be used to form even bands of the sample across the thin layer. The technique is of practical interest in micropreparative separations and the chromatographic behaviour of coloured compounds applied across the whole width of the plate has therefore been investigated. This study permitted the formation and separation of the bands to be observed during development of the chromatograms.

EXPERIMENTAL

The details of the construction of the tank (a modification of the Brenner–Niederwieser tank for continuous development) have been given previously[3,4]. The main feature of the tank is that the solvent is delivered by a capillary siphon to a narrow slit which distributes the solvent rapidly across the whole width of the plate. The samples can be spotted behind the solvent front so that solvent demixing and pre-adsorption effects are eliminated and the chromatographic process corresponds virtually to equilibrium conditions[6], in contrast to those obtained in saturated tanks[7,8]. A single cover-plate can be used when the sample is applied in this manner. Pre-coated plates were used [silica gel Si 60 (10 × 20 cm) or silica gel Si 60 with a concentrating zone (20 × 20 cm); E. Merck, Darmstadt, G.F.R.]; the plates were activated at 125° for 1 h.

In preliminary experiments, R_F *versus* solvent composition relationships were determined for a number of azo dyes and other coloured compounds, using mixtures of *n*-heptane and methyl ethyl ketone (MEK) as developing solvents. The samples were spotted behind the solvent front to secure equilibrium conditions[6]. The R_F *versus* MEK concentration plots are shown in Fig. 1a and the corresponding R_M *versus* log (MEK concentration) plots, which are more suitable for graphical analysis[9,10], are shown in Fig. 1b. The results indicate that suitable retentions of the test solutes are obtained at a *ca.* 10–20% concentration of the polar solvent.

The test mixture of the coloured solutes was obtained by dissolution of aliquots in a 10 or 20% *n*-heptane solution of MEK, in accordance with the principle that in

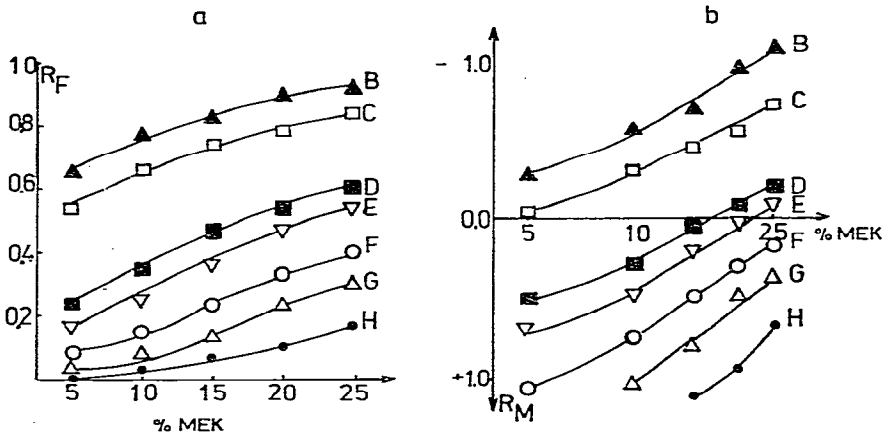
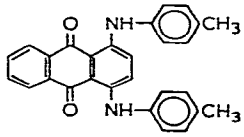


Fig. 1. Effect of concentration of methyl ethyl ketone (diluent: *n*-heptane) on R_F and R_M values. For notation of solutes see text.

preparative separations the sample should be dissolved in the developing solvent[11]. The concentrations of the solutes (in order of decreasing R_F values) were as follows: A, azobenzene, 0.25%; B, Fast Green B, 0.05%; C, Sudan IV, 0.01%; D, Sudan III, 0.01%; E, 1-aminoanthraquinone, 0.01%; F, 4-aminoazobenzene, 0.025%; G, 4-nitroaniline, 0.25%; H, methyl red, 0.01%.



Fast Green B

The sample solution was carefully introduced into the slit between the distributor and the margin of the plate, which was cleaned of the adsorbent, (in the shape of a horizontal 0.3 × 6 × 98 or 0.3 × 6 × 198 mm flat pipette) with a 0.5-ml syringe which permitted the volume of the solution to be determined. The following modifications of development and band application procedures were used:

(a) the sample solution was applied on the dry layer;

(b) pre-development was carried out with the developing solvent to a distance of 6–12 cm followed by application of one distributor volume of the sample solution and development.

To secure even, parallel bands it was necessary to apply the stop-flow technique at each change of the solvent under the distributor. When the flow of liquid into the tank is stopped, the thin layer of the liquid under the distributor is gradually depleted at the two ends and the area of the film diminishes towards the centre of the plate (Fig. 2a–c), causing the formation of a concave crescent-shaped front. This unfavourable phenomenon was eliminated by shifting the distributor 2–3 mm further over the thin layer (Fig. 2d). The narrow space (*ca.* 0.1 mm) between the distributor and the thin layer was then filled with the liquid until it was completely depleted and the resulting flow was strictly uniform across the whole width of the

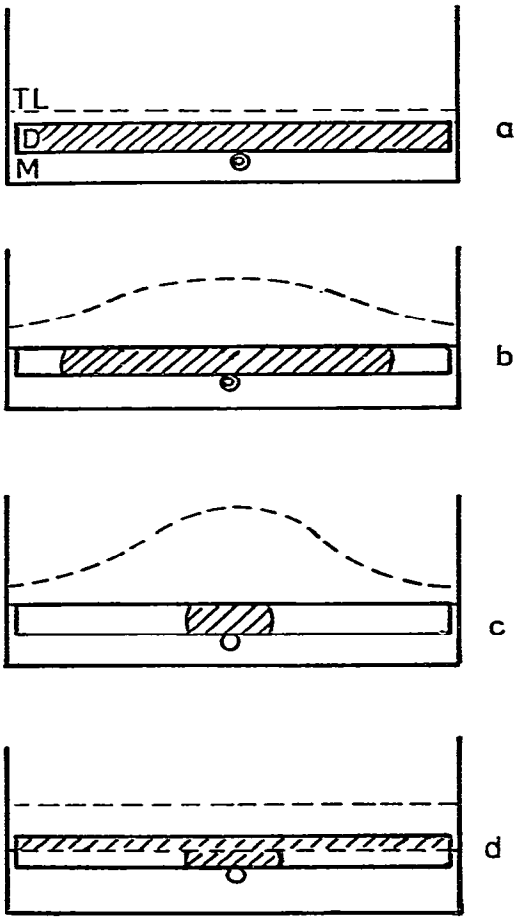


Fig. 2. Top view of the distributor (D) relative to the thin layer (TL); a, b and c, solvent flow uneven due to depletion of solvent (shaded area) under the distributor D; d, even flow for partial overlapping of the distributor and the edge of the adsorbent layer (TL). M, margin of the plate with the adsorbent removed.

plate. Thus, a typical chromatographic experiment consists of the following steps (Fig. 3):

(1) Pre-development of the layer (to eliminate solvent demixing, which would lead to merging of the leading zones): the slit under the distributor is filled with developing solvent in position a* and the cover-plate with the distributor is shifted to position b. The distance of development necessary for the solvent demixing front to leave the system before being overtaken by the fastest component of the sample depends on the composition of the developing solvent (see ref. 6 for detailed information).

* The first portions of the liquid are absorbed by the dry layer very rapidly so that the liquid under the distributor can be partly depleted (as in Fig. 2b). This effect can be eliminated by holding the container slightly higher for a few moments in order to accelerate the solvent flow.

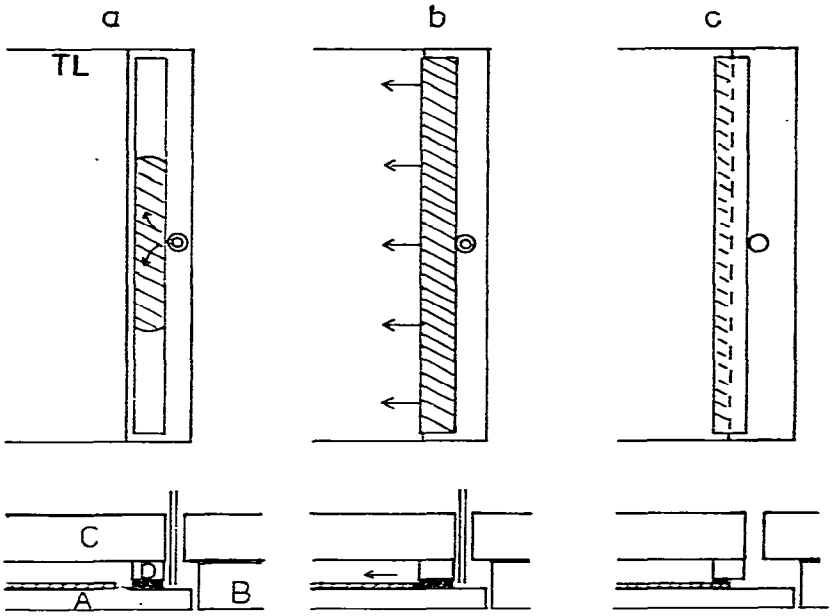


Fig. 3. Three positions of the distributor D relative to the edge of the thin layer TL, top view and cross-section (schematic, expanded 4:1 in vertical direction): a, formation of a thin film of liquid under the distributor; b, development, the liquid film is brought into contact with the layer; c, position securing an even flow of solvent. A = Carrier plate; B = spacing plate; C = cover plate.

(2) Application of the sample solution. The capillary siphon is removed from the orifice in the cover-plate (position c). After depletion of the solvent under the distributor, the cover-plate is shifted back to position a and the slit under the distributor is filled with the sample solution from a container with a capillary siphon, from a pipette or from a syringe. For the equipment used the volume of the solution was about 200 μl (for 10 × 20 cm plates). The cover-plate is then carefully shifted to bring into contact the sample solution and the thin layer (position b), and then to position c. The solution is absorbed by the layer as an even band.

(3) Development. The cover-plate is shifted back to position a, the slit filled with the developing solvent and development continued in position b.

It is essential for the formation of even bands that the leading edge of the distributor and the edge of the thin layer should be straight and smooth. For pre-coated plates the adsorbent tends to flake off and it is necessary first to draw a line with a sharp needle, to scrape off the margin and to smooth the edge. No side margins were necessary.

It was found that for very thin distributor plates (*e.g.*, 0.6 mm, as in ref. 4) the solvent tends to be drawn further into the narrow space between the cover-plate and the thin layer, causing an uneven flow. Therefore, 1.25-mm distributors (produced by cutting glass carrier plates of Merck pre-coated TLC plates into 5-mm strips) were used. The spacing plates were then 2.9 mm thick (1.25 + 0.4 + 1.25 mm).

RESULTS AND DISCUSSION

The following modifications of the procedure were investigated:
(a) pre-development with the developing solvent;
(b) Use of plates with a weakly adsorbing zone;
(c) variation of solvent composition.

Schematic representations of the chromatograms obtained are given in Figs. 4–6. The development was carried out until the leading zone approached the end of the layer.

Fig. 4a represents the separation of solutes applied on the dry layer (without pre-development). Zones B and C are merging and their separation is poor owing to a steep solvent demixing gradient[6]. Apart from some edge effects, the coloured zones are regular and most of the solutes can be eluted in a pure form; even the closely adjacent zones of solutes B and C are satisfactorily separated, which could be observed owing to their different colours. The effect of merging of the leading zones caused by solvent demixing would have been even stronger for mixtures containing several weakly polar solutes which would require development with a more dilute polar solvent.

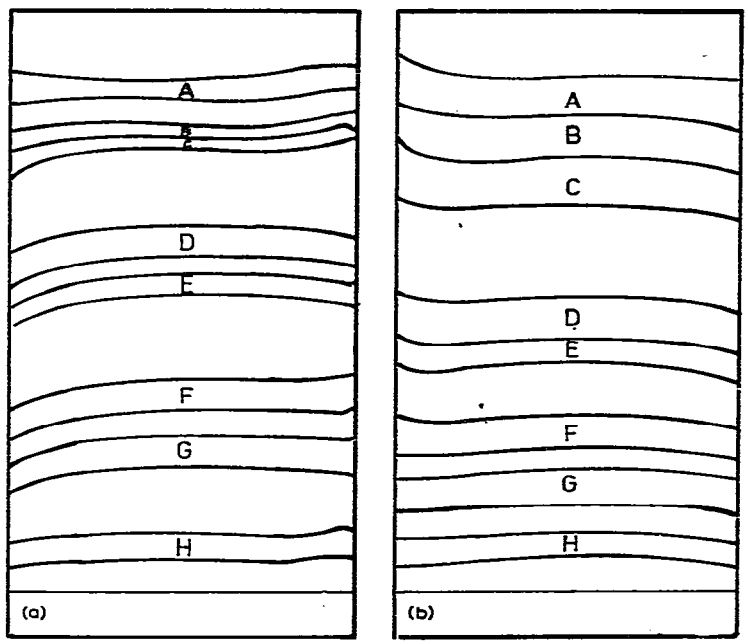


Fig. 4. Chromatograms obtained when 250 μl of test mixture (dissolved in 10% MEK) were applied on the dry layer (a) and after 6 cm of pre-development (b). Developing solvent: 20% MEK in *n*-heptane.

To eliminate this effect, in further experiments the sample was applied on the layer after pre-development with the solvent (Fig. 4b), which resulted in the formation of better separated, although more diffuse zones of B and C.

In other experiments the effects of sample size and layer thickness were investigated. For a 0.5-mm layer the zones were sharper and better separated; for

thicker layers a good separation was also obtained with double the sample size, whereas for thinner layers doubling of the sample size resulted in wide zones and marked deterioration of the separation due to overloading.

Fig. 5 illustrates the effect of solvent composition. It can be seen that the range of optimal solvent strength is narrow: for 5% MEK partial separation of G and H is obtained, while for concentrations exceeding 25% of the polar solvent the less polar solutes form overlapping zones.

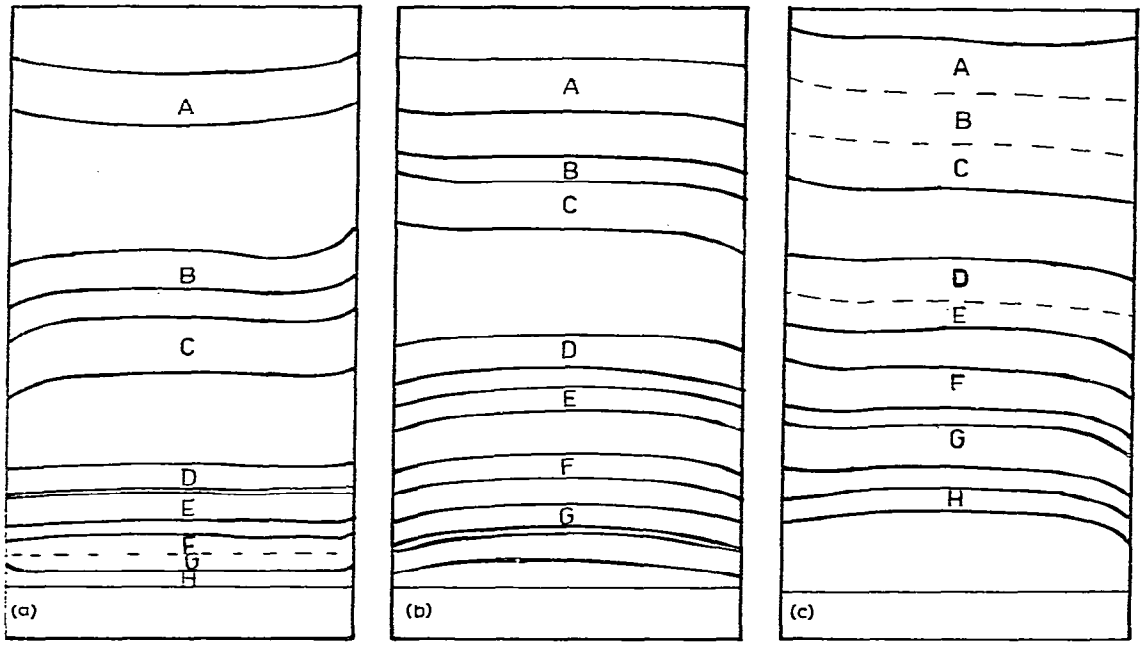


Fig. 5. Effect of solvent composition on the separation of 250 μl of test mixture: (a) 12 cm pre-development, 5% MEK; (b) 9 cm pre-development, 10% MEK; (c) 6 cm pre-development, 25% MEK.

Very even 20-cm bands were obtained using pre-coated plates with concentrating zones (Fig. 6). The plates have a 2.5-cm zone covered with large-pore silica gel of very low specific surface area, for which the solutes migrate with the front of the developing solvent and are thus concentrated on the adjacent edge of Si 60 silica gel as a very thin, concentrated band[12]. This improves the separation during further development. The sample size could easily be doubled without impairing the separation.

The results indicate that the inexpensive equilibrium sandwich tank with a glass distributor can serve to produce long, even bands of separated components suitable for micropreparative separations. To avoid merging of the leading zones owing to the formation of steep composition gradients (solvent demixing), it may be necessary, especially for low-concentration solvents, to pre-develop the plate before formation of the sample band[6]. Pre-development is also advantageous for the removal of impurities adsorbed from the laboratory atmosphere by the thin layer[13]; for the preparation of very pure samples for sensitive physico-chemical investigations

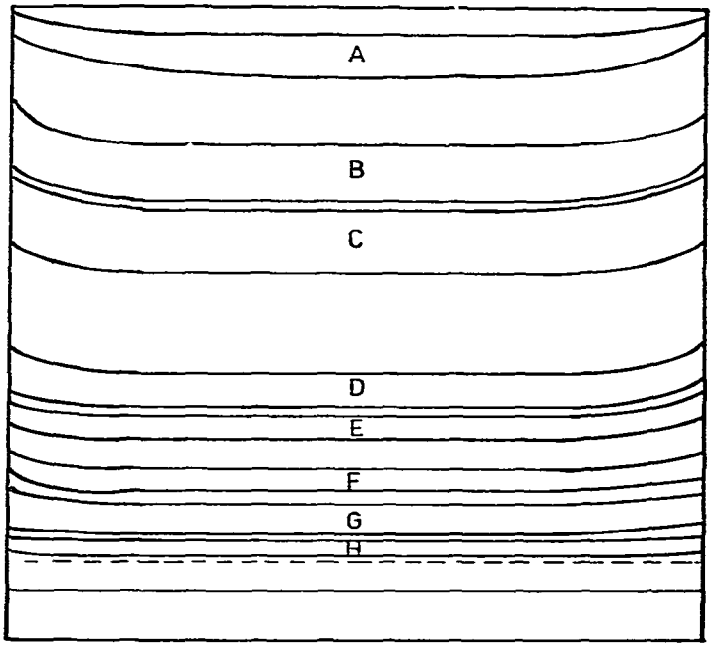


Fig. 6. Separation of test mixture on 20 × 20 cm Si 60 plate with weakly adsorbing zone; 500 µl of test mixture of double concentration (in 20% MEK) applied after 9 cm of pre-development with 10% MEK and developed with 10% MEK.

it may be necessary to wash the layer with a suitable solvent by development in another sandwich tank just before the separation. The complete removal of the impurities from the plate may be facilitated by the use of a paper wick pressed on to the end and protruding from the tank.

As stressed previously[4], it is essential that the distance between the distributor and the margin of the carrier plates is constant (0.40 mm). This requires the use of carrier plates of constant thickness (± 0.01 mm). This requirement is easily fulfilled with pre-coated plates or by the use of glass carrier plates obtained by cleaning of used pre-coated plates (1.25 mm thickness). The solvent level in the container should be slightly lower than the thin layer, otherwise gravitational flow of the solvent leads to flooding of the tank. As is typical for sandwich tanks, the consumption of solvent is very limited; for instance, for 20 × 20 cm plates with 0.25-mm layers pre-developed to half the length only a few millilitres of the developing solvent were required.

As the technique described requires some preliminary training of the operator, those interested are advised to repeat some of the experiments with a mixture of coloured compounds of the type used in this study. Initial problems can then be easily observed and eliminated. Many colourless compounds can be detected under UV light, and therefore a typical procedure would consist in the determination of R_F *versus* composition plots using narrow plates and spotting the sample solution as single spots. After the choice of a suitable solvent composition the separated zones can be localized under UV light and extracted from the scraped-off adsorbent. It may be advantageous first to remove the adsorbent along the edge where the zones

are sometimes irregular. For adjacent zones it may be necessary to scrape off a wider dividing line to discard overlapping fractions; however, owing to mutual displacement the boundaries between the zones are usually well defined, as judged from inspection of the chromatograms of coloured solutes. After the formation of dividing lines the localized zones can be pre-concentrated by development at right-angles to the first direction with a volatile solvent of high eluent strength. It is recommended to use developing solvents of the type used in high-performance liquid chromatography, that is, volatile UV-transparent solvents of low viscosity. The chromatographic system can then be used directly also in column chromatography.

It should be emphasized that in the technique described the mixture begins to separate during the application from the distributor (conditions of frontal analysis) as the direction of flow of the solution is identical with that during development. To secure a narrow starting zone it may be advantageous to introduce the sample in a solvent of lower elution strength (low R_F values) if the solubility of the solutes is sufficient[11]. Larger sample volumes can then be applied. On the other hand, the use of a more polar medium for the sample may cause irregularities owing to a local "reversed" solvent composition gradient and even temporary precipitation of some solutes owing to dilution of their concentrated solution by the mobile phase. This results in the formation of multiple streaks along the chromatogram and a poorer separation.

The optimal conditions and variations of the development technique depend on the mixture to be separated, the number of components, their proportions, etc. In some instances even the solvent demixing observed on the application of the sample on a dry plate may be advantageous owing to considerably flattened zones in the proximity of the demixing front.

REFERENCES

1 E. Soczewiński and K. Czapińska, *J. Chromatogr.*, 168 (1979) 230.
2 E. Soczewiński, *Chemia Anal. (Warsaw)*, 23 (1978) 515.
3 E. Soczewiński and J. Kuczmierczyk, *J. Chromatogr.*, 150 (1978) 53.
4 E. Soczewiński and T. Wawrzynowicz, *Chromatographia*, 11 (1978) 466.
5 W. Gołkiewicz and E. Soczewiński, *Chromatographia*, 11 (1978) 454.
6 T. Wawrzynowicz and E. Soczewiński, *J. Chromatogr.*, 169 (1979) 191.
7 P. A. Bristow, *Liquid Chromatography in Practice*, hetp, Wilmslow, 1976, p. 261.
8 A. Zlatkis and R. E. Kaiser (Editors) *HPTLC — High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977, p. 139.
9 E. Soczewiński, *Chromatographia*, 11 (1978) 534, and references cited therein.
10 S. Hara, Y. Fujii, M. Hirasawa and S. Miyamoto, *J. Chromatogr.*, 149 (1978) 143.
11 J. J. De Stefano and J. J. Kirkland, *Anal. Chem.*, 47 (1975) 1193A.
12 H. Halpaap and K.-F. Krebs, *J. Chromatogr.*, 142 (1977) 823.
13 M. Wotschokowsky, *Kontakte*, No. 1 (1975) 14.