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Migration of Zones of Test Dyes in Preparative Thin-Layer Chromatography: Stepwise Gradient Elution

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Abstract

Migration and separation of zones of four test dyes were recorded and compared for isocratic elution and five-step gradient elution in a sandwich chamber with a glass distributer. The maximal load capacity for full separation of all components of the sample was several times larger for stepwise gradient elution.

In a preceding paper (*1*) the formation and migration of zones in thin-layer chromatography were investigated under conditions of overloading (effect of sample size and its dilution). The experiments were planned as model experiments for column preparative chromatography. The technique of equilibrium sandwich chromatography (*2*, *3*), in which the sample is introduced from the edge of the layer (frontal + elution chromatography) (*1*, *4*), permitted the observation of migration and separation of zones in the chromatographic systems.

Gradient elution has occasionally been employed in preparative column chromatography. It has been observed in TLC separations of complex samples (plant extracts) that gradient elution markedly improves the separation of spots or zones owing to the stronger displacement effects within the initial zone; it seems that strongest displacement effects take place under conditions of numerous adsorption-desorption processes of individual solute molecules, that is, for moderate k' values (*5*, *6*). The gradually increasing eluent strength of the mobile phase passing through the partly separated starting zone causes the consecutive sample components to reach the optimal range and are arranged in the order of increasing k' values; thus, in spite of wide starting zones (e.g., 1-2 cm; partly separated in the frontal chromatography stage) (*1–3*), the separated zones are quite narrow (*5*, *6*).

In the present study the behavior of a four-component mixture of dyes was investigated. The mixture was applied in the form of wide zones to increase the sample capacity; stepwise gradient or isocratic elution was applied under analogous conditions to compare the separation capacities of the two elution modes. Since the zones of the component zones were visible, it was possible to record their migration and separation during the elution process, as described in the preceding study (*1*).

EXPERIMENTAL

50 × 200 mm glass plates were covered with 0.5 mm layers of silica (Si 60, E. Merck, Darmstadt, F.R.G.) using a Jobling-Quickfit spreader; the layers were dried and activated in the usual manner. Four dyes were investigated: 4-chlorobenzene-1-azo-1-4(*N*,*N*)-dimethylaminobenzene, Polanildunkelblau 3 RT, Polanilrubin FL, and Polanilscharlach RR. The dyes were dissolved in 10% solution of ethyl acetate in trichloroethylene. The sample solution contained 0.1% concentration of each dye (total 0.4%).

The chromatograms were developed in sandwich chambers with a glass distributer (*2*, *3*) which was also used to apply the sample zones from the edge of the layer (*1*, *3*, *4*). To eliminate solvent demixing effects, the layer was predeveloped to $R_F = 1.0$ before the application of the starting band.

RESULTS AND DISCUSSION

The sample solution band applied from the edge of the layer had the form of a frontal chromatogram. Isocratic elution was carried out with a single void volume (~1.3 cm^3) of 30% ethyl acetate in trichloroethylene; in gradient elution, five 0.2 mL portions of eluent of increasing concentration of ethyl acetate were introduced consecutively under the distributor (a linear gradient: 10, 20, 30, 40, and 50% EtOAc in trichloroethylene) (*6*). Azobenzene was used as the marker of the migration of the mobile phase ($R_F \approx 1.0$); the passage of its spot from the start to the end line corresponded to the elution with a single void volume.

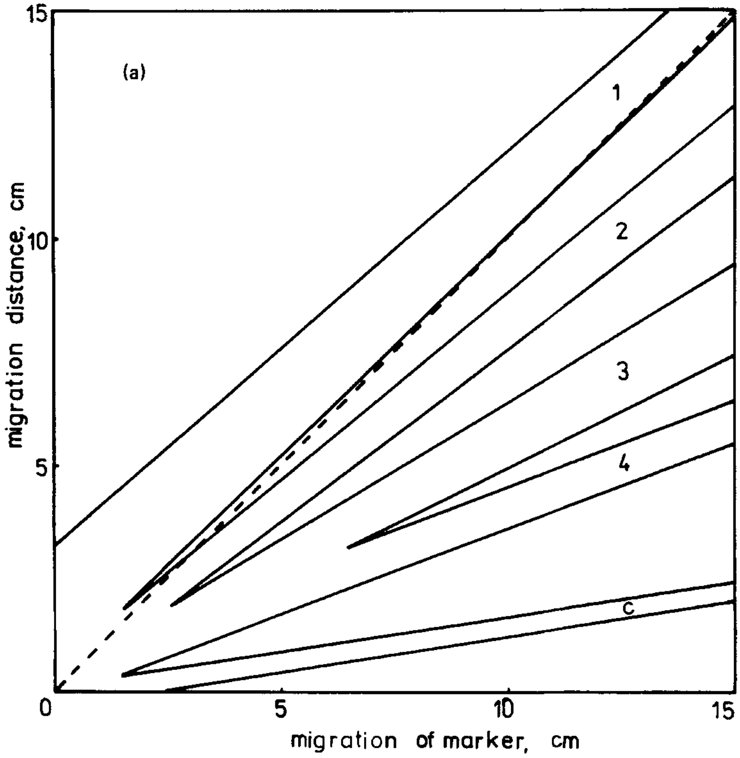
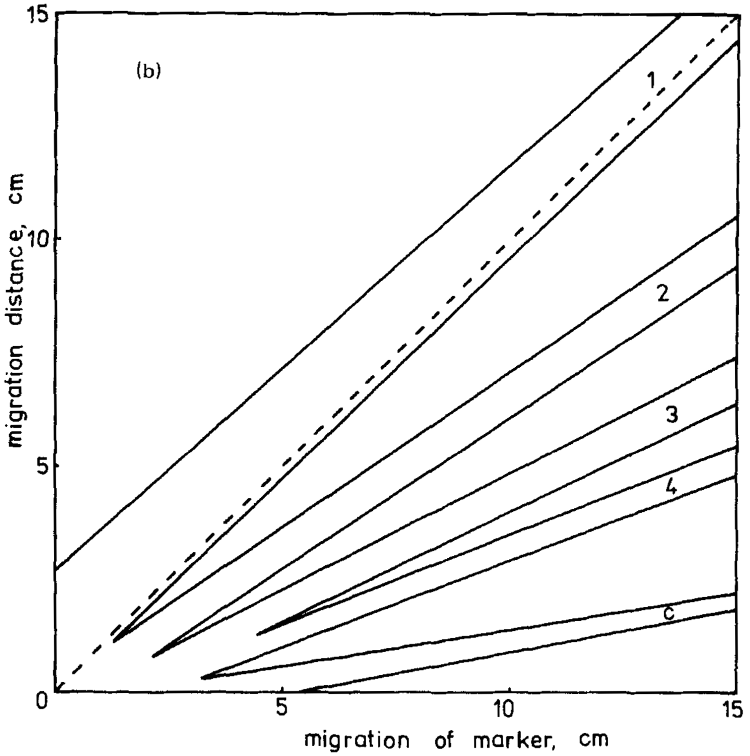


FIG. 1. Dynamic representation of migration of the bands of four test dyes. Sample size: 0.5 cm^3 of 0.4% solution of 4-chlorobenzene-1-azo-1-4(*N*,*N*)-dimethylaminobenzene (1), Disperse blue—Polanildunkelblau 3RT (2), Disperse red—Polanilrubid FL (3), and Disperse red—Polanilscharlach RR (4); c, contamination of No. 4. The dashed line represents the migration of the marker, azobenzene. (a) Isocratic elution with 30% ethyl acetate in trichloroethylene. (b) Five-step gradient elution, 10-20-30-40-50% ethyl acetate in trichloroethylene.



The movement of the zones relative to that of the marker spot was recorded visually on a transparent foil as described in the preceding paper (*1*).

Figures 1–3 illustrate the separation of the sample during isocratic elution for increasing sample sizes (0.5, 1.0, 1.5 cm^3); the lowest zone represents a contamination of one of the dyes. It can the seen that full separation is obtained for 0.5 cm^3 sample size; for 1 cm^3 the zones of dyes 2 and 3 partly overlap and the separation is still worse for the 1.5 cm^3 sample (Fig. 3a). It should be noted that the starting bands are relatively wide (R_F of the leading edge: 0.2–0.4).

For the simple stepwise gradient elution, the zones are more compact. Instead of spreading, the leading zones become even narrower during their migration. In consequence, the sample capacity is markedly higher—full separation of all components is observed even for 1.5 mL sample size. The procedure thus permits the complete separation of a 6-mg sample on a 0.5 × 50 × 150 mm layer.

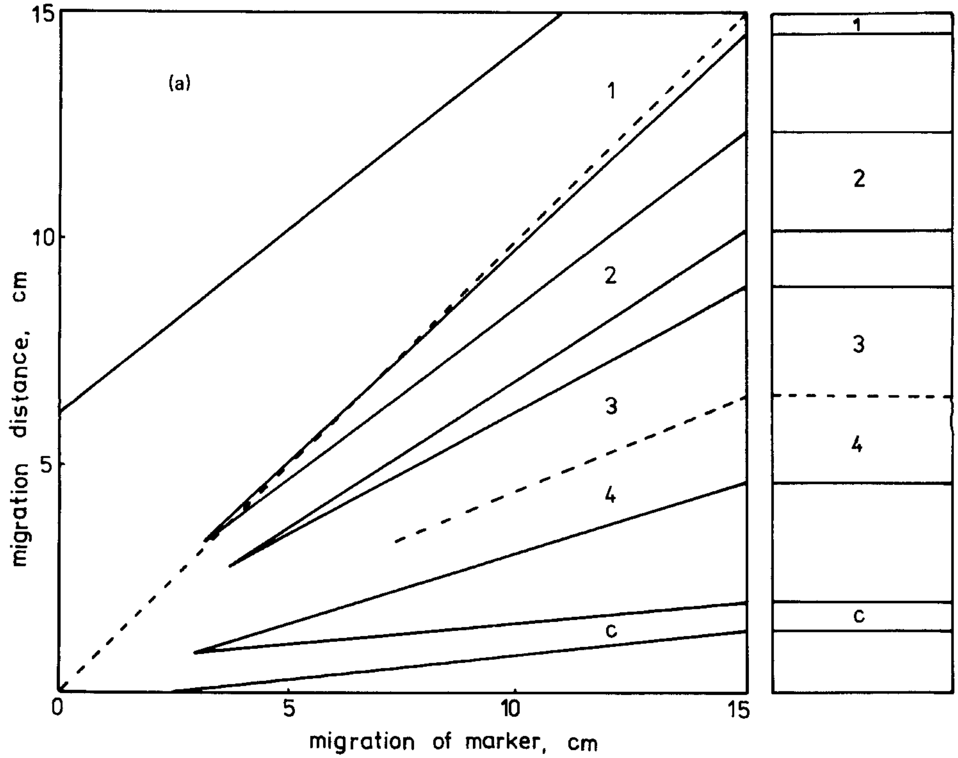


FIG. 2(a). As in Fig. 1. Sample size: 1 cm^3 of 0.4% solution of the dyes. Corresponding band chromatograms of dyes 1–4 are shown.

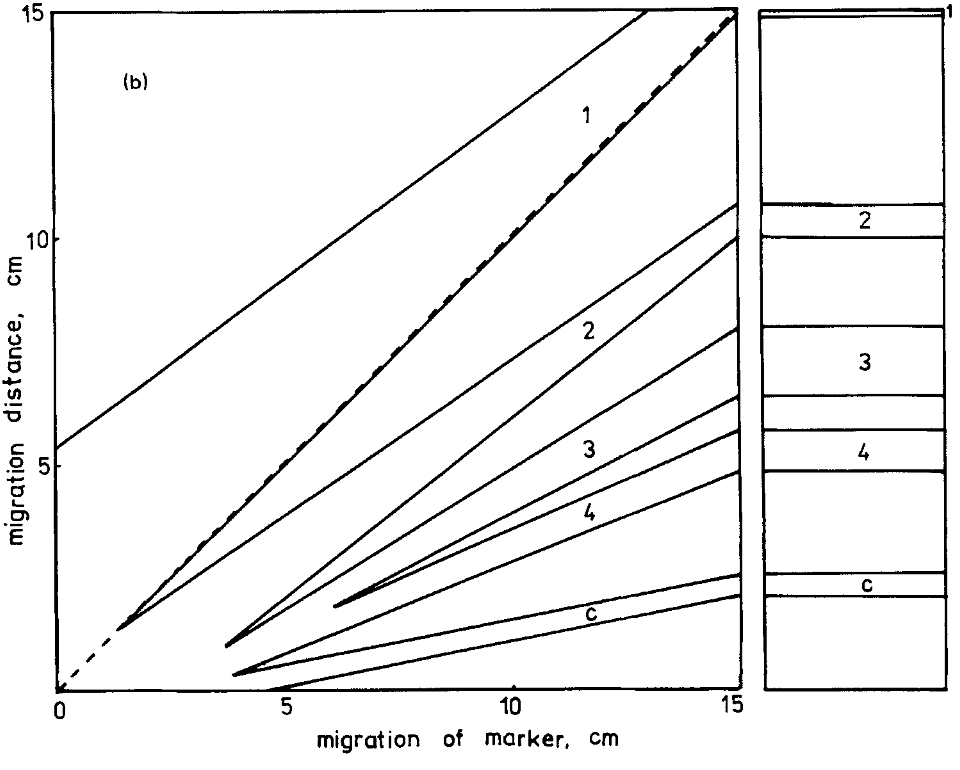

(b)
1
2
3
4
c
1
2
3
4
c
0
5
10
15
5
10
15
migration distance, cm
migration of marker, cm

FIG. 2(b).

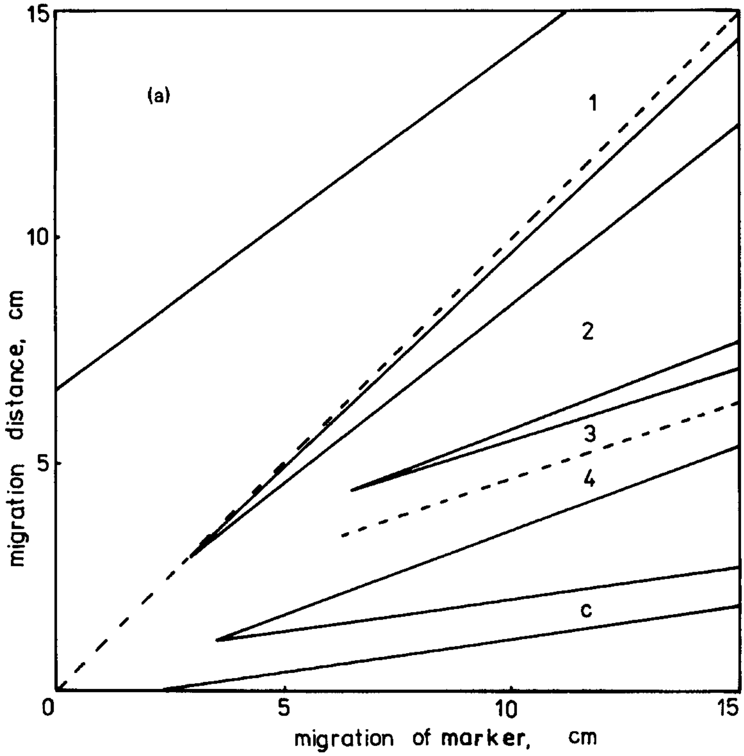
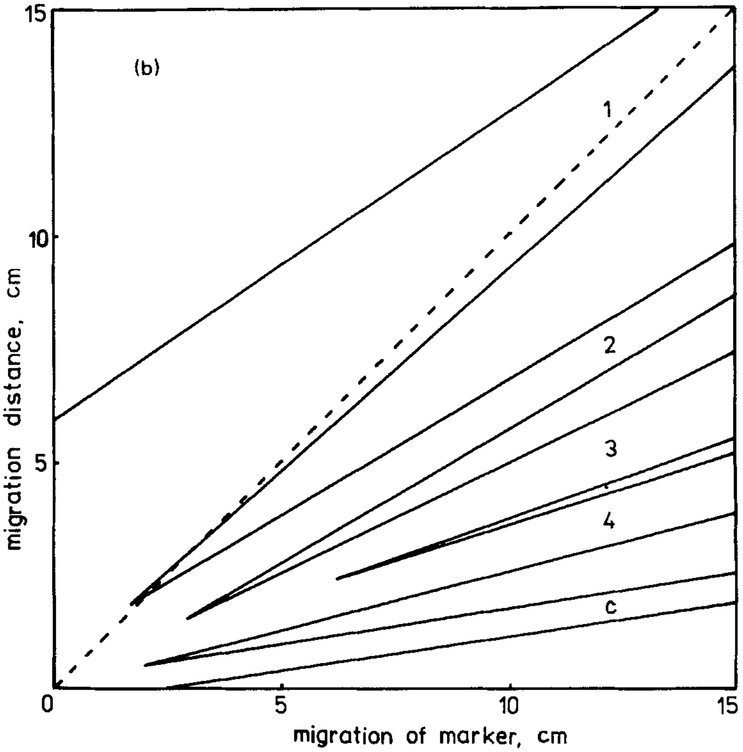


FIG. 3(a). As in Fig. 1. Sample size: 1.5 cm^3 of 0.4% solution of the dyes.


(b)
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c
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0
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10
15
migration distance, cm
migration of marker, cm

FIG. 3(b).

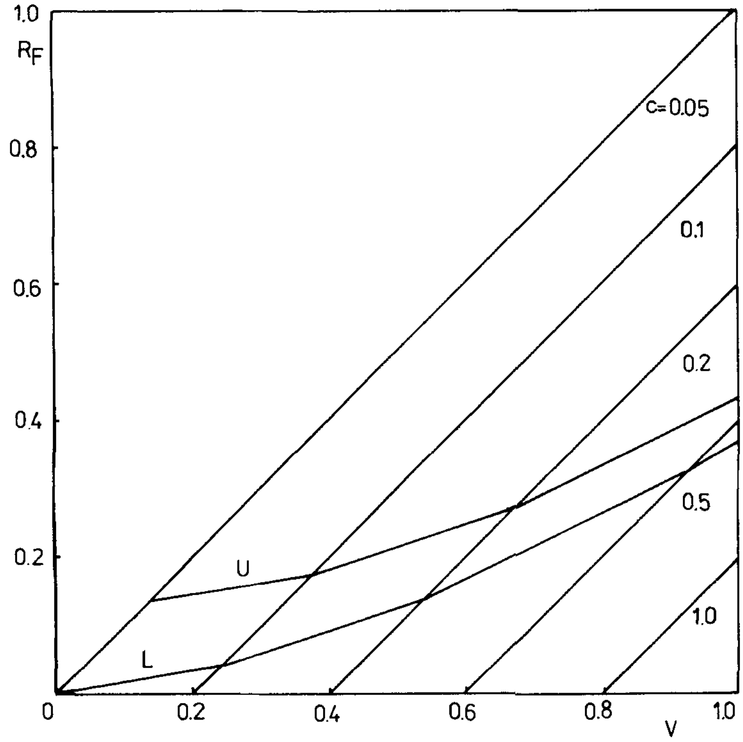

FIG. 4. Compression of a zone under conditions of five-step gradient elution (5–100% modifier concentration): the upper edge (U) is overtaken by the fronts of increased modifier concentrations (ascending lines) with delay relative to the lower edge of the zone (L).

The improvement of separation under conditions of stepwise gradient is caused by two mechanisms: mutual displacement enhanced by the gradient (*3*, *5*) and compression of the zone, described earlier for continuous gradients (*7*, *8*). For stepwise gradients the interpretation of the zone compression effect is straightforward (Fig. 4; cf. Ref. *9*): the lower edge of the zone is overtaken by the fronts of mobile phase of increased eluent strength earlier than the upper edge. Thus, the upper edge of the zone is accelerated by each consecutive front of the mobile phase with some delay.

Five-step gradient elution is relatively simple (*6*). It requires the introduction with a micropipet of five fractions of eluent (according to a chosen program) under the distributor. The improved separation and increased sample capacity may make the slightly more complex procedure worthwhile.

REFERENCES

1. T. Wawrzynowicz, E. Soczewiński, and K. Czapińska, *Chromatographia, 20,* 223 (1985).
2. E. Soczewiński and T. Wawrzynowicz, *Ibid., 11,* 466 (1978).
3. E. Soczewiński, in *Planar Chromatography,* Vol. 1 (R. E. Kaiser, ed.), Hüthig, Heidelberg, 1986, p. 79.
4. E. Soczewiński, B. Psionka, and J. Kuczmierczyk, *J. Liq. Chromatogr., 3,* 1829 (1980).
5. E. Soczewiński, G. Matysik, and K. Glowniak, in *Instrumental High Performance Thin-Layer Chromatography, Proc. 3rd Int. Symp., Würzburg IV, 1985* (R. E. Kaiser, ed.), Institute for Chromatography, Bad Dürkheim, 1985, p. 413.
6. G. Matysik and E. Soczewiński, *J. Chromatogr., 369,* 19 (1986).
7. P. Jandera and J. Churaček, *Gradient Elution in Column Liquid Chromatography,* Elsevier, Amsterdam, 1985.
8. L. R. Snyder, J. W. Dolan, and J. R. Gant, *J. Chromatogr., 165,* 3 (1979).
9. E. Soczewiński and W. Markowski, *Ibid., 370,* 63 (1986).