The sample solution band (test dye mixture), applied from the edge of the layer, formed a partly separated starting zone (frontal chromatography stage). After adsorption of the sample by the adsorbent layer, the eluent was introduced under the solvent distributor, and the marker (azobenzene) was spotted. The movements of the marker and the dye zones were recorded on a transparent foil (97). By connecting the points representing the upper and lower boundaries of the zones, a dynamic picture of the movement and separation of the zones could be obtained.

Stepwise gradient elution has been applied to the overloaded zonal preparative TLC of complex, multicomponent plant extracts of the herbal medicines azulan and hemorigen (98) used in therapy. Stepwise gradient elution combined with application of extract from the edge of the layer markedly improved the separation efficiency and purity of fractions, which was revealed by densitometry.

Theoretical and practical problems related to computer-aided optimization of stepwise gradient development in TLC of plant extracts containing biologically active compounds were reviewed by Matysik and Soczewiński (99).

Figure 14 (24) illustrates the separation of a dye sample during (a) isocratic and (b) stepwise gradient elution. It can be seen that full separation is obtained only for gradient elution; in isocratic elution, zones of dyes 3 and 4 overlap.

In the case of a stepwise gradient, the zones, instead of spreading, become narrower and more compact. In consequence, the sample capacity is markedly higher. The improvement of separation in preparative stepwise gradient elution is caused by two mechanisms: mutual displacement of the components of the mixture to be separated and compression of the zones, described earlier for continuous gradients in HPLC (1,4,5). The compression of the zones results from the fact that the lower edge of a zone is overtaken by the mobile-phase fronts of increasing eluent strength earlier than the upper edge, so that the upper edge of the zone moves in the mobile phase of a lower eluent strength than the lower edge.

VI. CONCLUSIONS

Gradient development can be applied for the following purposes:

Separation of samples that contain many compounds with widely different retention values
Lowering of the detection limit by sharpening of the chromatographic zones
Speeding up the search for a better chromatographic system

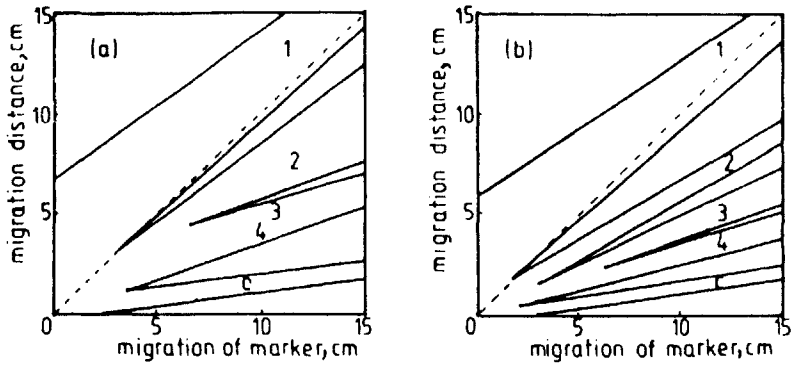

Figure 14 Dynamic representation of the migration of the bands of four test dyes. Sample: 1.5 mL of a 0.4% solution of 4-chlorobenzene-1-azo-1,4(*N*,*N*)-dimethylaminobenzene (1); disperse blue-Polanildunkelblau 3RT (2); disperse red-Polanilrubid FL (3); and disperse red-Polanilscharlach RP (4); c, contamination 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% of ethyl acetate in trichloroethylene. (Reprinted from Ref. 24 with permission.)