the individual components in the developing solvent; it increases with an increase in concentration of the stronger solvent. The height and steepness of the gradient and length of the zones depend on the nature and the concentration of the eluent components. [The gradient steepness for linear gradients can be expressed as the percent per minute change in the concentration of solvent B (Fig. 2) or, for nonlinear gradients, as the average percent per minute change in the concentration of solvent B.]

A developing solvent that contains n components will give n zones separated by $n - 1$ fronts. In polyzonal TLC, it is of particular interest to vary the distance between the immersion line and the starting point of the mixture. This can be done by applying the mixture solution several times at different distances from the immersion line. Any changes in the mobile and stationary phases during chromatography influence the behavior of the solute, depending on the distance between the starting point and the immersion line. As can be seen in Fig. 7, the complete chromatographic separation of a complex mixture can often be conveniently carried out by the use of two or more different starting points. Spots 4 and 7 from the first starting point (first mixture from the left side) are not separated, although spots 8 and 9 are well separated. The situation is different for the second starting point: Spots 8 and 9 are not separated, in contrast to 4 and 7.

During the chromatographic process, molecules of each new solvent displace the solvent molecules of lower eluent strength and push the demixing front nearer to the α front. Generally, it is advisable to create conditions that allow these fronts to spread in equal proportions over the entire development distance (6).

The greater the differences between the components of the mixture to be separated, the greater must be the range of solvent strengths of the components of the eluent.

In general, mixtures in equimolar amounts of the lower representatives of any homologous series are frequently used. The following mixtures are useful (6):

Chlorinated hydrocarbons: carbon tetrachloride–chloroform–methylene chloride (96:80:64)
Ethers: diisopropyl ether–diethyl ether–dioxane (141:104:85)
Esters: *n*-butyl acetate–*n*-propyl acetate–ethyl acetate–methyl acetate (132:115:98:80)
Ketones: cyclohexanone–diethyl ketone–methyl ketone–acetone (103:106:90:73)
Alcohols: *n*-butanol–*n*-propanol–ethanol–methanol (92:75:58:40)

Polyzonal TLC with a multicomponent mobile phase represents the simplest technique for stepwise gradient elution. A continuous gradient can be realized only if the eluent contains a great number of different components with very small increments in solvent strength. However, because

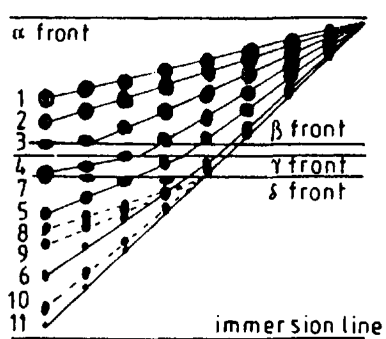

Figure 7 Polyzonal chromatogram of a mixture containing, in 0.5 μL, 1 μg each of the 2,4-dinitrophenyl derivatives of the following amines and amino acids: (1) *n*-amylamine, (2) *n*-butylamine, (3) *n*-propylamine, (4) ethylamine, (5) methylamine, (6) tyramine, (7) leucine, (8) methionine, (9) proline, (10) hydroxyproline, and (11) glutamine, with separation starting from eight increasingly higher origins. Silica gel G (Merck), air-dried layer (relative atmospheric humidity, 50%), BN chamber, solvent isopropylether–propionic acid–acetic acid–formic acid (100:0.66:0.66:0.66). (Reprinted from Ref. 30 with permission.)