

values obtained under stepwise gradient conditions. After introduction of R_f values of the sample components obtained for several isocratic runs, the microcomputer calculates R_f values for any gradient program and displays the paths of migration of the spots through the concentration zones. It is thus possible to study by computer simulation the final arrangement of spots for chosen programs of stepwise gradients.

2. Automated Multiple Development

Optimization of gradients in automated multiple development (AMD) can be achieved in three steps:

1. Selection of the "base" solvent (i.e., medium polarity) and at least two modifiers (very polar and nonpolar solvents)
2. Improvement of the separation by development of a final gradient with the appropriate range of eluotropic strengths of the solvent mixtures
3. Development of a suitable slope of a gradient (i.e., rate of change of the eluotropic strength with time)

Solvents with the selectivity necessary for the separation of the mixture are usually selected (57,58) with the help of the PRISMA model, based on Snyder and Kirkland's (76) solvent selectivity scheme. Selection of the correct base solvent from the different Snyder classes turned out to be critical to the optimization of selectivity.

The eluotropic strength of the binary solvent mixtures can be calculated using Snyder's equation (77).

When the individual components of the mixture to be analyzed are available, preliminary experiments based on isocratic development may be useful for selection of suitable solvents. The preliminary investigation may be performed as follows (56). The retention behavior of high and medium polarity standards in binary mixtures of strong and "base" solvent is carried out to determine the solvent strength range of the AMD gradient. Successive investigations using binary mixtures composed of the base solvent and (usually) hexane are carried out to optimize the separation of low polarity standards. The isocratic data obtained for different concentrations of binary mixtures are conveniently plotted as the relationship between R_m and solvent composition (9–12). Inspection of these plots gives useful information about the adequate solvent strength and the change in selectivity resulting from the change of base solvent and modifiers.

If the polarity range of an AMD gradient is such that insufficient resolution is obtained, the separation might be optimized by changing the gradient slope. Queckenberg and Frahm (58) stated that, in general, steeper gradients improve peak shape but reduce the resolution, whereas flatter gradients generate broader but better separated peaks.

Two gradient profiles are recommended: universal (56,61) and linear (58,59). Some authors (58,59) prefer a linear gradient because abrupt changes in eluotropic strength occur within the universal gradient (59), and some components of a complex mixture might coelute. The concentration of mobile phase at which the coelution occurred corresponded to an abrupt change in the eluotropic strength, thus explaining the results observed (59).

The optimization procedure is frequently carried out by the trial-and-error method (56–60,67) owing to the lack of a theoretical model of the multiple development process. Markowski and Soczewinski (78,79) formulated the physical model for AMD, which is useful for describing the migration of the solute zones and computer analysis of various parameters determining the final optimization of gradient.

Let us consider two-step gradient development (80). After a first development to the distance z_1 , the R_f of the solute is equal to

$$y_1 = z_1 R_{f1}$$

where R_{f1} is the R_f value for the first eluent. The chromatogram is now dried and developed to distance z_2 with the second eluent, for which the solute R_f is equal to R_{f2} . However, the spot does not move until the solvent front overtakes it; thus, the real solute migration distance in the second step is $z_2 - z_1 R_{f1}$. The final R_{fg} value for the two steps of gradient is