$$R_{fg} = \underset{\text{first development}}{z_1 R_{f1}} + \underset{\text{second development}}{(z_2 - z_1 R_{f1}) R_{f2}}$$

Generalizing the situation for an n-step gradient, we can write

$$R_{fg} = \sum_{i=1}^{h=(n-1)} y_i + y_n = \sum_{i=1}^{h=(n-1)} y_i + \left(z_n - \sum_{i=1}^{h=(n-1)} y_i \right) R_{fn}$$

where R_{fg} is the final R_f value after the n-step gradient, $\Sigma_{i=1}^{h=(n-1)} y_i$ is the sum of the preceding fractional migration distances, y_n is the real R_f value in the last step, z_n is the development distance in the last step, and R_{fn} is the isocratic R_f value of the solute for the solvent used in the last step.

A computer program for the calculation of the final R_{fg} value, taking into account the development distances z_i, compositions of consecutive eluents, and the retention–modifier concentration relationship, was elaborated by Markowski (79).

V. GRADIENT ELUTION IN ANALYTICAL AND PREPARATIVE TLC

As demonstrated in many papers (23,81–83), much better separation efficiency is obtained for stepwise gradient elution than for continuous elution, especially in the case of plant extracts, owing to enhanced displacement effects.

Matysik and Jusiak (82) used stepwise gradient development for the separation of chelidonium alkaloids in waste industrial fractions. Binary (toulene–methanol) and ternary (toulene–ethyl acetate–methanol) mobile phases were used, and a six-step program was performed. Eight-step stepwise gradient elution was also used for separation of glycosides from *Digitalis* species (83).

Ergot alkaloids (84) and coumarin derivatives (85) were separated on TLC silica plates by using stepwise gradients with different solvents. Stepwise gradients have also been used to separate anthocyanins (86) in the petals of red poppy, furocoumarins (87), and anthraquinones (88).

Marked improvement of the separation of two plant extracts by the use of a modified program of stepwise multiple gradient development was reported (89). Modification lies in the fact that the chromatographic plate was developed over decreasing distances with eluents of increasing eluent strength.

Gradient development combined with densitometry is an efficient method for the analysis of plant extracts, because it eliminates preliminary purification of extract. Examples of such a procedure are presented in some papers, e.g., perstilbene (3,5-dimethoxy-2-hydroxy-*E*-stilbene) was satisfactorily separated by use of two-step gradient elution and quantified by densitometric techniques (90). In another work (91), plant extracts containing flavonoids were separated on HPTLC silica plates by two- and three-step gradient elution.

An HPTLC method with densitometric detection was used to determine the convallatoxine content of extracts from flowers, leaves, and underground parts of *Herba convallariae* (92). Plant extracts were separated on HPTLC silica plates by multiple gradient development.

Mycotoxins such as alternariol and alternariol methyl ether, produced by fungi of the genus *Alternaria*, were analyzed by stepwise gradient TLC (93). The obtained chromatograms were well suited for quantitative densitometric determination.

Two-step gradient elution was applied to separate the colored pigments of *Trichoderma harzianum* fermentation broth (94). The main fractions were identified by instrumental methods (IR, DAD detector, and MS) after gradient reversed-phase TLC. Additionally, multistep gradient elution developed for RP-TLC was successfully used as a pilot method for the rational design of a gradient elution program in RP-HPLC.

Fluorescein, the active component in the French preparation "fluoresceine," was quantitatively determined after gradient HPTLC development (95). Gradient mobile-phase TLC was also applied to the quantitative determination of prednisolone acetate in a Polish preparation "prednisolon" and in the aqueous humor of rabbit eyes (96).

Gradient development has occasionally been employed in preparative TLC chromatography. Soczewinski and coworkers (24,97) applied an equilibrium sandwich chamber (47) for systematic investigations of the formation of zones and separation selectivity in overloaded preparative liquid chromatography.