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## Gradient Development in Thin-Layer Chromatography

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### I. INTRODUCTION

The separation of multicomponent mixtures by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) under fixed experimental conditions is often complicated by large differences in the polarity of the various components. To deal with this problem, eluents of low strength are needed to separate the less strongly retained solutes, whereas the strongly retained components of the mixtures can be separated by eluents of high strength. This is referred to as the general elution problem (1), and in TLC it can be handled in various ways: gradient elution (stepwise or continuous), stationary-phase gradient, polyzonal TLC, or temperature programming. These various techniques are based on different band migration rates of the components of the mixture during the separation process.

Gradient development in liquid chromatography stands in contrast to isocratic elution, in which the conditions of separation are not changed throughout the time required for the sample separation. In gradient development the situation is different: The conditions of separation (mobile-phase concentration, composition of the adsorbent layer, temperature, etc.) are changed during the separation. These continuous or stepwise changes in the separation conditions lead to changes in the relative migration velocity of the components of a sample. For example, if the concentration of the stronger solvent in a binary mobile phase increases, the eluent strength and  $R_f$  values of all solutes are also increased. As a result, separate optimization of the  $R_f$  values of individual bands is possible.

Gradient development in TLC is a technique that allows one to improve the resolution of a given pair of adjacent bands, to accelerate a separation, to concentrate the sample band and lower the detection limit, and to speed up the search for an optimal chromatographic system.

Successful separations of many complex mixtures by HPLC gradient elution have demonstrated the utility of this technique (1–5). In contrast to HPLC, gradient development in TLC has been applied relatively rarely, owing to the rather complex devices required for the generation of reproducible gradients and the lack of a simple theory of gradient development. Niederwieser and Honegger (6,7) systematized many experimental results and outlined some theoretical problems.

Recently, gradient development in TLC has become more popular, as evidenced by papers on theory (6–15), devices for gradient development (16–23), and the preparative mode (24).

The purpose of this chapter is to acquaint the reader with the most popular gradient techniques in TLC, including their characteristics, advantages, and limitations.

### A. History of Gradient Development in TLC

The idea of using gradient development in column chromatography is ascribed to work by Tiselius and coworkers in 1952 (25), but as early as 1949, Mitchell et al. (26) used salt and pH gradients for the separation of some enzymes.