

As was shown in Section II.A, adsorbent gradients can be achieved in several different ways. For example, a strong adsorbent (e.g., silica gel) is mixed with varying proportions of a weak adsorbent (e.g., kieselguhr). As a result, an adsorbent gradient is formed along the plate. In fact, gradients composed of silica gel and kieselguhr have not fulfilled expectations. Greater dilution of the silica gel with kieselguhr (or other adsorbent of low surface area) results in reduced capacity and overloading of the initial part of the plate (31).

Layers containing a discontinuous adsorbent gradient usually consist of a narrow zone of adsorbent A along the lower edge of the plate and an adsorbent B on the remaining part of the plate [layers with five zones of different adsorbents were also proposed (53)]. Discontinuous adsorbent gradients are used for three purposes:

1. To adsorb some interfering components of the sample at the starting point (31,32). The adsorbent in zone A strongly retains the unwelcome substances, e.g., an ion-exchange of complexing mechanism, but it does not retain the rest of the components of a mixture.
2. To carry out two-dimensional TLC. In the first direction, isocratic TLC occurs along the zone of adsorbent A. In the second direction, prefractionated sample components enter the layer of adsorbent B, which differs as much as possible from adsorbent A, for example, in pH or the presence of a complexing agent (31).
3. To concentrate the spot applied on a narrow preconcentration zone of a very weak adsorbent (e.g., kieselguhr). During development by an eluent, the spot is concentrated into a narrow band because the solvent strength is too high for such a weak adsorbent.

Many examples of continuous and discontinuous adsorbent gradients applied in practice are given by Niederwieser (31) and by Liteanu and Gocan (3).

The adsorbent layer can also be exposed to solvent vapors in special sandwich-type chambers that permit various solvent vapors to contact different parts of the plate, resulting in an adsorbent activity gradient along the plate. This technique is called preloading (43) or vapor-programmed gradient TLC (40).

If the chromatographic plate is exposed to the vapors of a strong solvent such as acetone, the adsorbent layer is highly deactivated and high R_f values are obtained. The opposite effect would occur for a weak solvent such as hexane. A vapor-programmed gradient can also be applied either parallel to the direction of solvent flow or at right angles to it (for details, see Ref. 49). This method of gradient generation is relatively simple. However, the actual composition of the adsorbent layer and the gradient shape are virtually unknown.

E. Automated Multiple Development

Perry et al. (54,55) introduced in 1973 a new technique called programmed multiple development (PMD), in which the TLC plate was repeatedly developed in the same direction with the same solvent. Burger (17) improved this technique but maintained the general principles of PMD. The Burger (17) method is called automated multiple development (AMD). The characteristics of the AMD system are as follows (17–19):

1. A TLC plate is repeatedly developed in the same direction with solvents that differ from one step to the next.
2. Each developing step is longer than the previous one (approximately 3 mm per step).
3. From step to step, the solvent strength is decreased.
4. Gradient elution is used, but, in contrast to HPLC, the gradient starts with the most polar solvent (usually a mixture of methanol and dichloromethane, 50:50) and ends with the weakest solvent (e.g., a mixture of dichloromethane and *n*-hexane).
5. Solvent is completely removed from the plate after each developing step so that the composition of the solvent introduced in the next step is not changed.
6. From 10 to 25 steps are necessary to develop a plate, which corresponds to a total developing time of 0.5–3 h and a total migration distance of 3–10 cm.