

modifiers (e.g., water or acetic acid) for normal-phase OPLC. If the R_f values are too high (>0.8), the solvent may be diluted with hexane, provided it is miscible.

A statistical method for quantifying mobile-phase selectivity was developed for one- and two-dimensional OPLC separations, and it was applied for the separation of steroids (60a).

IV. APPLICATIONS

A. Possibility of Analytical Applications

1. Improvement of Resolution

Resolution in HPTLC is limited by the development distance, because it cannot be increased beyond 8–9 cm. Using OPLC as a forced-flow technique permits longer development distances, and the resolution can be significantly increased. The effect of longer development distance on the resolution can be seen in Fig. 13, where doping agents were separated from a mixture (61). Botz et al. (23c) had the developing distance further increased. By using a serial multilayer, the “long-distance” OPLC technique, they separated materials over a developing distance of more than 50 cm.

The main protein amino acids were separated in a double-layer system by Tyihák et al. (61a). The separation was performed on HPTLC silica plates that were serially coupled so the bed length was 340 mm. The mobile phase was *n*-butanol–acetonitrile–0.005 M KH_2PO_4 –acetic acid (10:5:30:10). The chromatogram was evaluated by densitometry at 490 nm after detection by ninhydrin reagent.

Empore™ silica sheets, because of their physical characteristics, cannot be used in a conventional chamber system over a 5 cm development distance (62). Due to the forced flow, OPLC makes a longer development distance and rapid separation possible on this sorbent. This is promoted also by the higher density of the sheet caused by overpressure (63).

The effectiveness of separation can also be improved with the OPLC technique by using different modified sorbent materials such as diol (64) and amino phases (65). Bis-indol alkaloids extracted from *Catharanthus roseus* were separated (Fig. 14) and determined on a laboratory-modified amino-bonded HPTLC silica gel sorbent (66). Optimization of the mobile phase was performed by the PRISMA model followed by factorial experimental design. Silica gel impregnated with tricaprilmethylammonium chloride (TCMA) was applied for separation of different groups of compounds using eluents containing methanol and water (67). The retention mechanism was not ion-pairing but hydrophobic interaction between the analytes and the caprylic groups of the TCMA.

2. Faster Development with Viscous Solvent Mixtures

Because of the forced flow, OPLC ensures a constant and high flow velocity, even in the case of viscous solvent mixtures with poor sorbent-wetting characteristics. For this reason, development time is significantly shorter than in TLC/HPTLC.

The classes of phospholipids were separated by using an *n*-hexane–2-propanol–water (40:53:7) eluent mixture. The time of development was only 20 min on 17 cm running distance (68).

Polar quaternary alkaloids in a plant extract were separated on a silica gel sheet in a distance of 14 cm; ethyl acetate–tetrahydrofuran–acetic acid (60:20:20) was used as the eluent (69). The development time was 10 min.

The development time was compared in the case of different eluents by using TLC and OPLC techniques for the separation of dinitrophenylhydrazones of saturated aldehydes and ketones (56). It was found that developing by OPLC was about 10 times faster in normal-phase systems and 5 times faster in reversed-phase systems than that in TLC.

The result was similar for the separation of organophosphorus warfare agents using diisopropyl ether–benzene–tetrahydrofuran–*n*-hexane (10:7:5:11) as eluent (58a). When the development distance was 1.25 cm, the developing time was 59 min by TLC and 9.5 min by OPLC.

Synthetic peptides were separated on a silica gel layer by an optimized eluent system (69a). The eluent was *n*-butanol–pyridine–acetic acid–water (12:4:1:4). The time of separation was