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INVESTIGATIONS AND RESULTS OBTAINED WITH OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

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SUMMARY

This paper investigates some separation performance data obtained under different conditions with a newly developed technique in the area of thin-layer chromatography, overpressured thin-layer chromatography (OPTLC). The changes in theoretical plate heights and resolution values for some lipophilic dyes were determined at different flow-rates, migration distances and layer thicknesses. Some of these data are compared with corresponding results obtained by linear high-performance thin-layer chromatography without pressure.

The advantages of OPTLC are particularly pronounced shorter migration times and higher separation efficiencies in the case of longer separation distances. In addition it is possible to adjust the flow-rates of the eluents for coping with special separation problems.

INTRODUCTION

The fundamental principles for extending the area of application of thin-layer chromatography (TLC) were laid down in previous years, particularly by the development of pre-coated layers with improved separation performance (high-performance TLC, HPTLC) as well as various surface-modified pre-coated layers (in some cases parallel to corresponding development in the area of high-performance liquid chromatography (HPLC)). Furthermore, various manufacturers of equipment have also developed instruments which meet the more stringent requirements of precision and reproducibility of sample application, chromatographic separation and evaluation in TLC.

The limited migration distances in HPTLC layers resulting from the relatively slow flow-rates and the not inconsiderable influence of the gas phase on the resulting separation led Tyihák and co-workers¹⁻⁴ to develop a new planar liquid chromatography technique, called overpressured TLC (OPTLC). The special feature of this method lies in the fact that the adsorbent layer of the pre-coated plate or foil is completely covered by a flexible membrane which is pressurized. The mobile phase is introduced into the thin-layer under pressure with the aid of a pump.

In TLC with linear ascending developments, the mobile phase is transported

through the layer by capillary forces and the flow constant, κ , is a quadratic function of time, t (eqn. 1)⁵

$$\kappa = \frac{z_f^2}{t} \quad (1)$$

where z_f is the migration distance. However, in linear OPTLC, assuming a constant-flow pump is used, a linear relationship exists between the migration distance, z_f , of the mobile phase and the time required (eqn. 2)⁶.

$$u = \frac{z_f}{t} \quad (2)$$

EXPERIMENTAL

Equipment

The following equipment was used: a Chrompres 10 overpressured thin-layer chromatograph from Labor MIM (Esztergom-Budapest, Hungary), and a fixed-volume Nano-Doser 100 nl (Pt-Ir capillary) from Camag (Muttentz, Switzerland).

Pre-coated layers

We used the following pre-coated layers: silica gel 60 F₂₅₄ HPTLC plates (E. Merck, Darmstadt, G.F.R.), silica gel 60 F₂₅₄ HPTLC aluminium foils (E. Merck), silica gel 60 F₂₅₄ HPTLC aluminium foils (40 × 20 cm), and silica gel 60 F₂₅₄ HPTLC aluminium foils with reduced layer thicknesses (50 and 80 μm).

Reagents and solvents

All reagents were of analytical-reagent grade, and the solvents were Li-Chrosolv grade (E. Merck).

Samples and sample quantities

A mixture of the following lipophilic dyes was used: ceres violet BRN, ceres black G, green constituent of ceres black G, fat yellow 3G, bleu VIF organol, ceres red G and ceres brown RRN. Toluene was used as the solvent. The concentration of the dyes was 0.1% in each case. The application volume was 100 nl.

Development

The pre-coated HPTLC plates and aluminium foils with silica gel 60 F₂₅₄ were developed in the Chrompres 10 instrument with migration distances of 5, 10, 15, 25 and 35 cm. The pump was set to supply the mobile phase at rates of 0.08–0.69 ml/min according to the exact setting. The resulting linear flow velocities were between 0.05 and 0.44 mm/sec.

Evaluation

The evaluation was carried out with a Camag TLC/HPTLC scanner (CAMAG, Muttentz, Switzerland).

RESULTS AND DISCUSSION

Dependence of the plate height, H , on the linear flow-rate u

As a constant linear flow-rate of the mobile phase is produced by the constant-flow pump in linear OPTLC, it is possible to record the relationship between the plate heights, H , of individual sample substances on the linear flow velocity (H - u curves), as in HPLC and gas chromatography.

The plate height, H , of a substance separated in a thin-layer chromatogram is calculated from the formula

$$H = \frac{w_x^2}{16z_x} \quad (3)$$

where w_x = peak base width of substance x and z_x = migration distance of the substance x from the start line to the peak maximum.

In Fig. 1, the plate heights, H , of some lipophilic dyes are plotted against the linear flow-rate, u , on silica gel 60 pre-coated HPTLC plates using a constant migration distance, z_f , of 15 cm. Toluene was used as the mobile phase. The curve confirms the results of Kalász and Nagy⁷, who found that the plate heights pass through a minimum value over a certain range of linear flow-rate, u . In this case, the range of flow velocity was *ca.* 0.18-0.26 mm/sec.

Relationship between the resolution, R_s , and the linear flow-rate, u

The resolution, R_s , of two neighbouring sample substances in a thin-layer chromatogram is calculated according to the formula

$$R_s = 2 \cdot \frac{z_{x2} - z_{x1}}{w_2 + w_1} \quad (4)$$

where z_{x1} = the migration distance of substance 1 from the start line to the peak maximum, z_{x2} = the migration distance of substance 2 from the start line to the peak maximum, w_1 = the peak base width of substance 1, and w_2 = the peak base width of substance 2.

Fig. 2 shows the curves for the resolutions, R_s , of several neighbouring lipophilic dyes as a function of the linear flow-rate, u , on silica gel 60 pre-coated HPTLC plates with a constant migration distance, z_f , of 15 cm. Here also, an optimal range of R_s values emerges at a linear flow velocity of between *ca.* 0.20 and 0.24 mm/sec. This range lies exactly in the area in which the minimum values were found in the relationship between plate heights and the linear flow velocity.

Relationship between the optimum plate heights, H_{opt} , (with reference to the linear flow-rate) and the migration distance z_f

In Fig. 1 it was demonstrated that the plate heights, H , pass through a minimum value, *i.e.* an optimum point, when the linear flow velocity, u , is varied, for a given migration distance, z_f . Fig. 3 shows the optimum plate height values, H_{opt} , obtained in this way on silica gel 60 pre-coated HPTLC plates with respect to the migration distance, z_f . Here it is noticeable that with increasing migration distance,

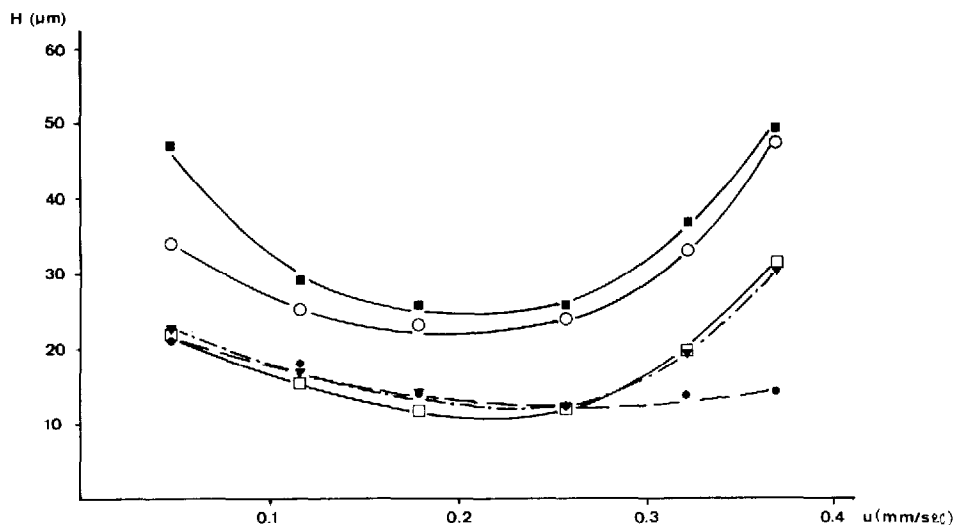


Fig. 1. OPTLC on silica gel 60 F₂₅₄ pre-coated HPTLC plates: plate heights, H , of a mixture of lipophilic dyes as a function of the linear flow velocity, u , of the eluent toluene; migration distance $z_f = 15$ cm: ●, ceres violet BRN; ▼, ceres black G; □, green component of ceres black G; ○, bleu VIF organol; ■, ceres red G.

z_f , the optimal plate heights decrease up to a migration distance of 25 cm and only increase slightly or remain constant at greater migration distances, in contrast to normal ascending TLC without pressure. With ascending TLC on silica gel 60 pre-coated HPTLC plates without use of pressure, using the same sample substances and the same eluent, the optimum migration distance for the optimal plate heights is only 4 cm⁸. This major difference can be attributed to the fact that in the case of OPTLC,

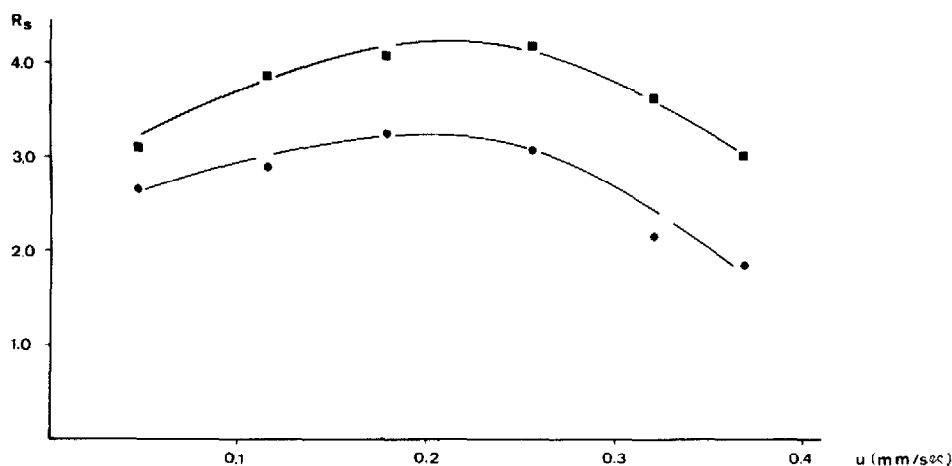


Fig. 2. OPTLC on silica gel 60 F₂₅₄ pre-coated HPTLC plates: resolution, R_s , of a mixture of lipophilic dyes as a function of the linear flow velocity, u , of the eluent toluene; migration distance $z_f = 15$ cm; ●, ceres black/green; ■, blue/ceres red.

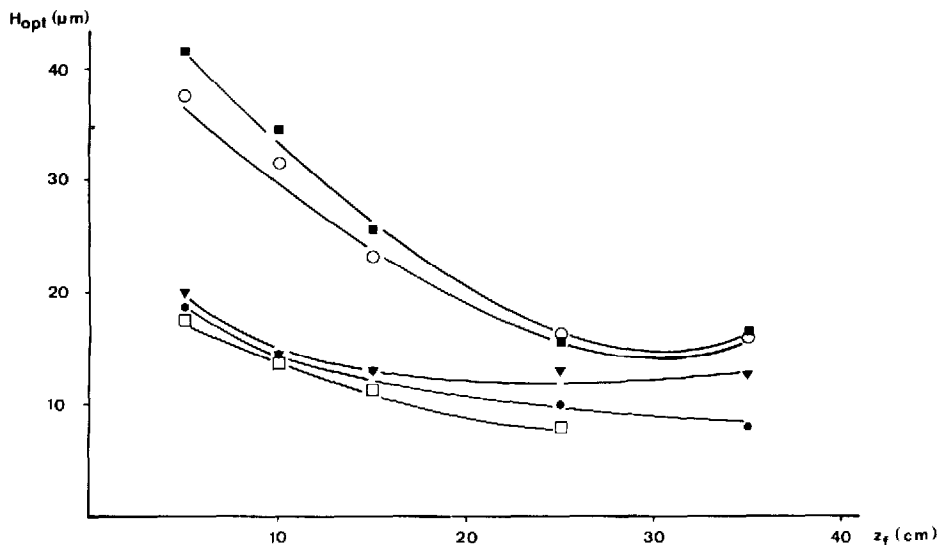


Fig. 3. OPTLC on silica gel 60 $F_{2.54}$ pre-coated HPTLC plates and aluminium foils: optimal plate heights, H_{opt} , of a mixture of lipophilic dyes (with reference to the linear flow velocity) plotted against the migration distance, z_f : \bullet , ceres violet BRN; \blacktriangledown , ceres black G; \square , green component of ceres black G; \circ , bleu VIF organol; \blacksquare , ceres red G.

the growth of the spot by diffusion processes plays a minor role even with longer migration distances because of the relatively high linear flow velocities and the resulting shorter development times. In contrast, in linear ascending TLC without pressure, a quadratic time law applies, with the result that the flow velocities decrease and the development times are longer, so that the enlargement of the spots due to diffusion is much more noticeable. The absolute magnitudes of the smallest attainable plate-height values for the sample substances and quantities used here in HPTLC on silica gel 60 pre-coated plates without application of pressure are, at *ca.* 18 μm for a migration distance of 4 cm ⁸, significantly higher than the results using the same HPTLC layers in OPTLC, *viz.* *ca.* 8 μm with a migration distance of 25 cm .

Relationship between the optimum resolution, $R_{s,opt}$ (with reference to the linear flow velocity), and the migration distance, z_f

The optimum resolutions, $R_{s,opt}$ of neighbouring lipophilic dyes, determined in the same way as the optimal plate heights with regard to the linear flow velocities, are plotted in Fig. 4 against different migration distances, z_f . Here it can be seen that there is an approximately linear increase in optimal resolution with increasing migration distance up to the maximum migration distance, z_f , of 35 cm possible with the experimental arrangement used.

The optimal values of $R_{s,opt}$ reach 9.1 for the two neighbouring dyes bleu VIF organol and ceres red G and therefore lie significantly above the R_s values obtained by linear HPTLC without use of pressure. These latter values were also determined for the separation example given and are shown in Fig. 4; they reach a value of $R_s = 2.88$ for a migration distance of 15 cm . The same applies to the resolution of the two

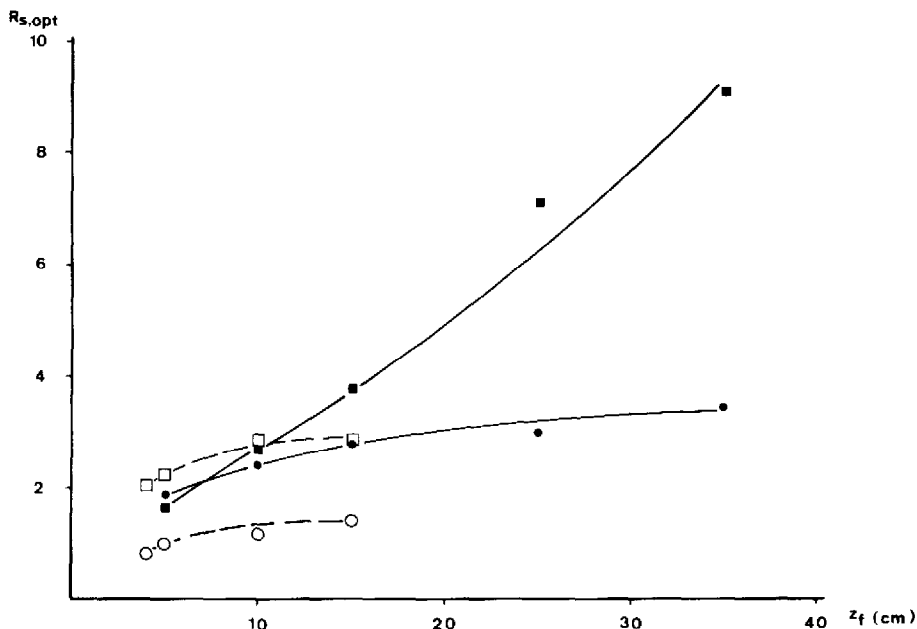


Fig. 4. OPTLC and HPTLC without application of pressure on silica gel 60 F_{254} pre-coated HPTLC plates and aluminium foils: optimal resolution, $R_{s,opt}$, of a mixture of lipophilic dyes (with reference to the linear flow velocity) plotted against the migration distance, z_f : ●, ceres black/green in OPTLC; ■, blue/ceres red in OPTLC; ○, ceres black/green in HPTLC without application of pressure; □, blue/ceres red in HPTLC without application of pressure.

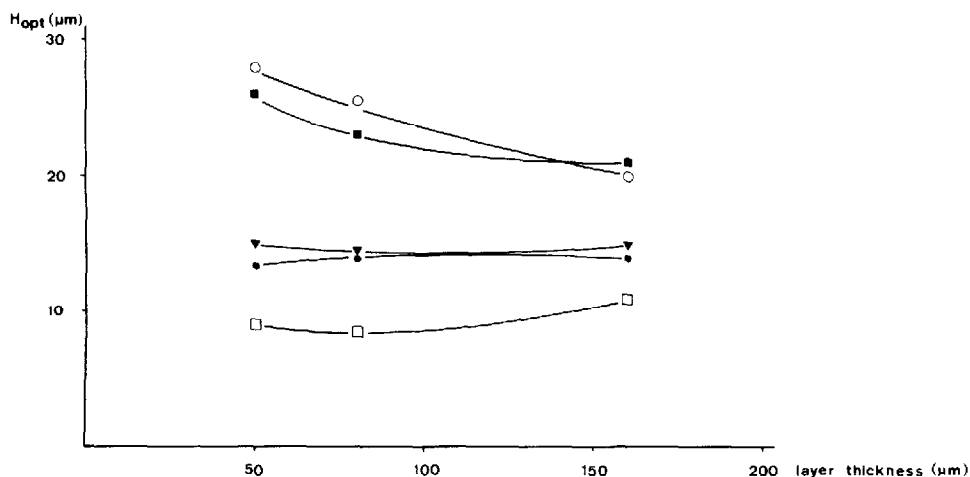


Fig. 5. OPTLC on silica gel 60 F_{254} HPTLC aluminium foils: optimal plate heights, H_{opt} , of a mixture of lipophilic dyes (with reference to the linear flow velocity) plotted against layer thickness; migration distance $z_f = 15$ cm: ●, ceres violet BRN; ▼, ceres black G; □, green component of ceres black G; ○, bleu VIF organol; ■, ceres red G.

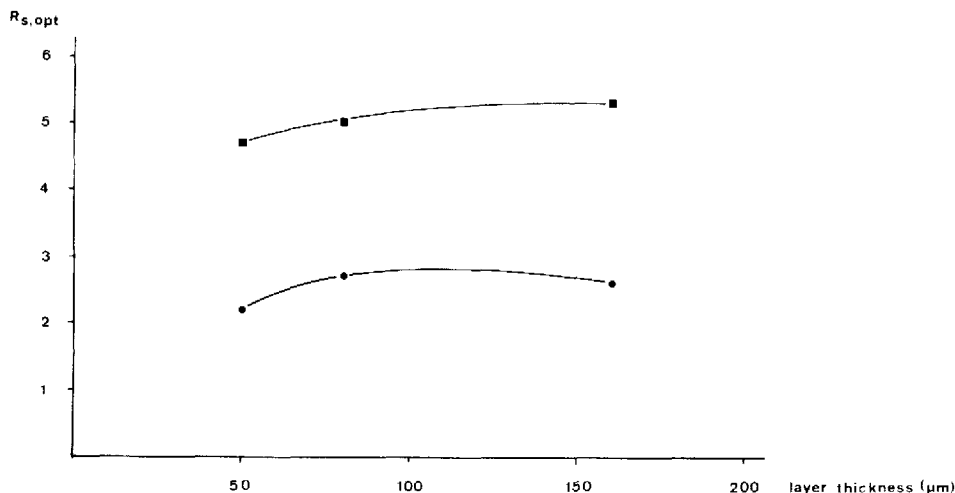


Fig. 6. OPTLC on silica gel 60 F₂₅₄ HPTLC aluminium foils: optimal resolutions, $R_{s,opt}$, of a mixture of lipophilic dyes (with reference to the linear flow velocity) plotted against layer thickness; migration distance $z_f = 15$ cm; ●, ceres black/green; ■, blue/ceres red.

dyes ceres black G and the green constituent of ceres black G which have values of $R_{s,opt} = 3.45$ and $R_s = 1.43$.

Relationship between the optimal plate height value, H_{opt} (with reference to the linear flow velocity), and the layer thickness

Several silica gel 60 F₂₅₄ HPTLC aluminium foils were prepared with different layer thicknesses (between 50 and 160 μm) under otherwise identical conditions. Fig. 5 shows that the optimal plate height values (with respect to the optimal linear flow velocities in each case) are most favourable at the selected migration distance, z_f , of 15 cm with layer thicknesses of the silica gel 60 material of between 80 and 160 μm (depending on the sample substance).

Relationship between the optimal resolution, $R_{s,opt}$ (with reference to linear flow velocity), and the layer thickness

In Fig. 6, the optimal resolutions $R_{s,opt}$, determined in the same way as the optimal plate heights, are plotted against the layer thickness of the silica gel 60 HPTLC layers used. Here too, it can be seen that, depending on the neighbouring pairs of dyes investigated, an optimum layer thickness is in the range 80–160 μm.

CONCLUSIONS

The results of the comparison of linear HPTLC without pressure and OPTLC with the same sorbent layers show the following advantages of OPTLC.

(1) OPTLC offers the possibility of optimizing separation conditions by adjusting the flow-rate of the eluent. This means a significant shortening of analysis times, in comparison with linear development without pressure, accompanied by a

pronounced smaller spread of spots (by influence of diffusion), especially in the case of eluents with high viscosities.

In addition to this, with the aid of OPTLC it is possible to force the migration of eluents in chromatographic systems with stationary phases of poor wettability by the mobile phase (*e.g.* pronounced hydrophobic reversed-phase layers and eluents with high water content). Therefore, in the case of these layers there is no longer any limitation in the selection of the solvents usable.

(2) In contrast to linear TLC without pressure (quadratic relationship between migration distance and time), in OPTLC there exists a linear law of time. Because of the pronounced shorter migration times, OPTLC has advantages especially when longer migration distances are used. This benefit of OPTLC can be utilized if substances with small differences in selectivity, or complex mixtures with a large number of compounds, have to be separated.

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