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RESOLUTION AND RETENTION BEHAVIOUR OF SOME DYES IN OVER-PRESSURED THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The performance of conventional and overpressured thin-layer chromatographic techniques was compared in different chamber systems (normal, ultramicro and pressurized chamber), on commercial normal and high-performance thin-layer chromatography (HPTLC) silica gel plates. Data obtained with three dyes demonstrated that separation in the pressurized chamber is similar to those observed in normal and ultramicro chambers, but the diameters of the spots are significantly smaller in separations carried out in pressurized chambers, principally owing to decreased diffusion. It was also observed that the velocity of the moving phase is very stable in the pressurized chamber, and has a linear relationship to solvent flow-rate.

Owing to the substantially shorter separation time and stable flow-rates in the pressurized ultramicro chamber, the resolution values obtained on HPTLC plates are also better in the case of longer solvent migration distances.

INTRODUCTION

During recent years, the problem of the optimization of separation conditions in thin-layer chromatography (TLC) has been tackled both in theoretical treatments¹⁻⁵ and in the development of instrumentation⁶⁻⁸.

We have developed a novel planar liquid chromatographic technique using a pressurized ultramicro chamber (PUM chamber^{9,10}). The essential feature of this chamber system is that, in contrast to the rigid glass plate used in the earlier UM chamber^{10,11}, the sorbent layer is completely covered by an elastic membrane under external pressure, so that the vapour phase above the layer is eliminated. Solvent is admitted into the chamber under overpressure by means of a pump system. This technique essentially combines the advantages of traditional TLC, modern high-performance TLC (HPTLC) and high-performance liquid chromatography (HPLC), leading to improved separation efficiency, time requirement, resolution, potential for column modelling, and solvent consumption, compared with classical TLC.

In this paper we report the comparisons of resolution and retention behaviour of some authentic dyes separated in classical and PUM TLC chambers, on commercial normal and fine-particle silica gel plates.

EXPERIMENTAL

Apparatus

Three chambers were used. (1) Normal Desaga TLC tank (N chamber). (2) Ultramicro chamber (UM chamber). This simple chamber is characterized by a glass plate covering the sorbent layer in such a way that the end of the plate is not immersed in the solvent, in order to eliminate interfering capillary effects^{10,11}. (3) Linear pressurized ultramicro chamber (PUM chamber)¹⁰.

In situ quantitative evaluation of the spots on the developed chromatograms was accomplished with a Zeiss PMQ II chromatogram spectrophotometer. Solvent admission into the PUM chamber was carried out with an Infumator (Kutesz, Budapest, Hungary).

Chromatographic plates

Pre-coated silica gel glass plates (conventional chromatographic plates) with indicator (silica gel 60 F₂₅₄, 200 × 200 mm; Merck Darmstadt, G.F.R.), without indicator (silica gel 60, 200 × 200 mm; Merck) and HPTLC silica gel 60 F₂₅₄ aluminium sheets (Merck) were used.

Chemicals

The Camag Test Dye Mixture I was used for separation. All chemicals were guaranteed reagent grade and were used without further purification.

RESULTS AND DISCUSSION

Comparisons of the overpressured and conventional TLC technique were carried out with normal, ultramicro and linear-type pressurized chambers, using both normal and HPTLC plates. Data obtained with three dyes in different chambers are shown in Fig. 1. It can be seen that the separation in the PUM chamber is similar to that in normal and UM chambers, but the diameter of the spots at longer solvent migration distances is much smaller, owing to the lower degree of diffusion (shorter running time), particularly on the HPTLC plate.

Fig. 2 presents the characteristics of solvent movement (distance against time) in the various chamber systems. In contrast to earlier studies¹⁰, we now compared the data obtained on normal and fine-particle layers. As may be seen on the figure and on the figure segment, no significant difference is observed between run parameters on normal and fine-particle sorbent plates in the case of short runs (e.g., 4.5 cm, the normal distance used in HPTLC⁶). At longer distances, however, longer times are required in normal and ultramicro chambers in the case of HPTLC plates, whereas in the PUM chamber a line was obtained on such layers at longer distances too, with satisfactory resolution.

Fig. 3 shows the behaviour of the three dyes in saturated and unsaturated N, UM and PUM chambers, using a one-component solvent and normal and fine-

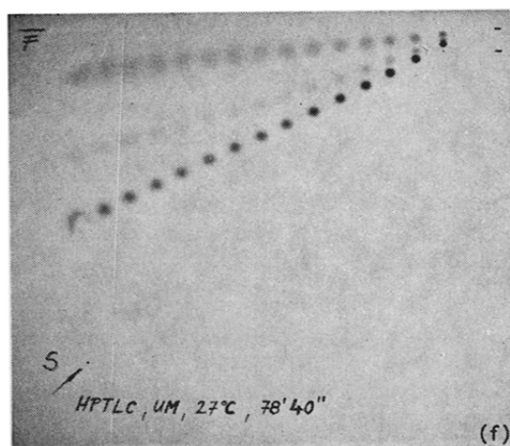
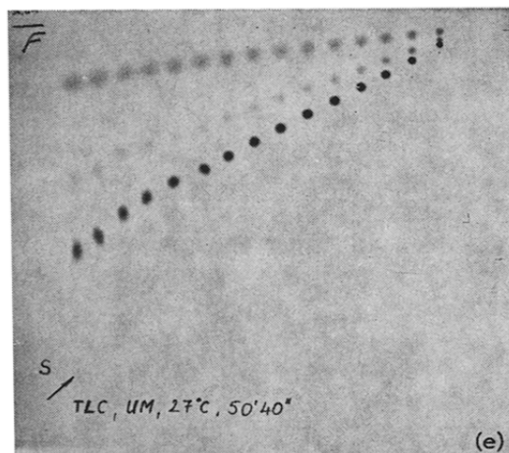
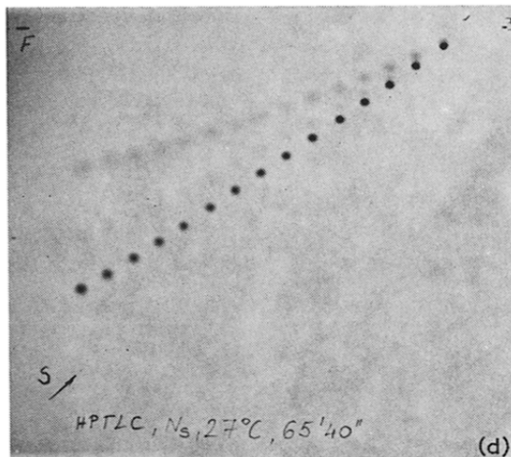
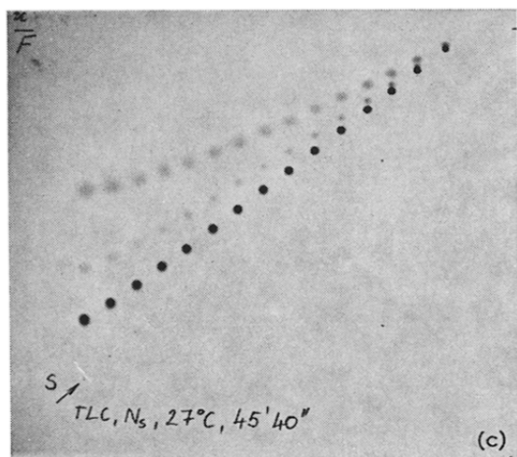
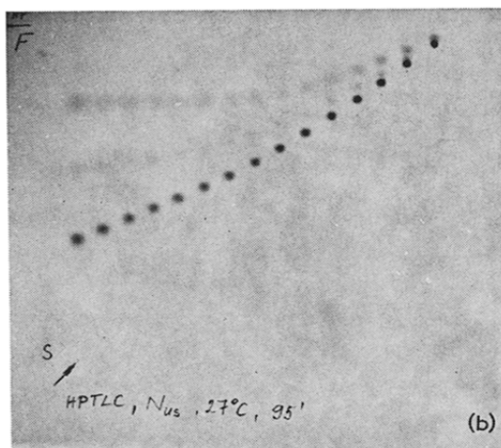
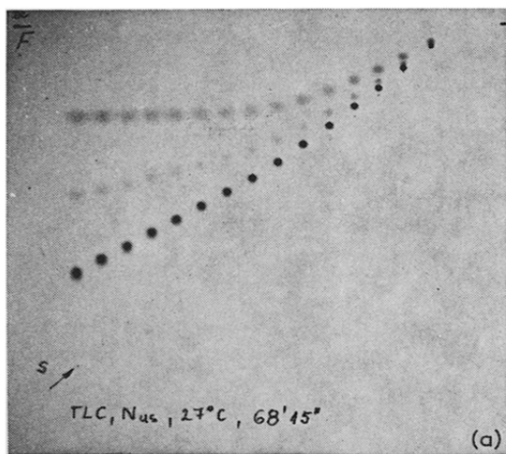


Fig. 1.

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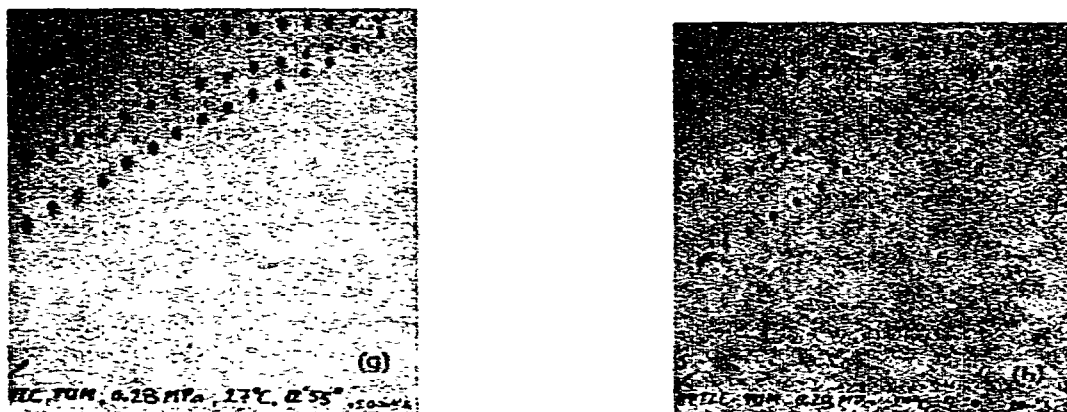


Fig. 1. Behaviour of the three dyes in saturated and unsaturated normal (N_n , N_{ns}), ultramicro (UM) and pressurized ultramicro (PUM) chambers. Sample, 2-2 μg Test Dye Mixture I in n -heptane (2 $\mu\text{g}/\mu\text{l}$); mobile phase, methylene chloride; sorbents, TLC aluminium sheets silica gel 60 F₂₅₄, 200 \times 200 mm (Merck); HPTLC aluminium sheets silica gel 60 F₂₅₄, 200 \times 200 mm; temperature, 27°; distance micratered, 170 mm. (a) N_{ns} chamber, TLC plate, 68.2 min; (b) N_{ns} chamber, HPTLC plate, 95 min; (c) N_n chamber, TLC plate, 45.3 min; (d) N_n chamber, HPTLC plate, 65.3 min; (e) UM chamber, TLC plate, 50.3 min; (f) UM chamber, HPTLC plate, 78.3 min; (g) PUM chamber, TLC plate, 12.9 min; external pressure on membrane, 0.28 MPa; flow-rate of solvent, 20 cm^3/h ; (h) PUM chamber, HPTLC plate, 13.3 min; external pressure on membrane, 0.28 MPa; flow-rate of solvent, 20 cm^3/h .

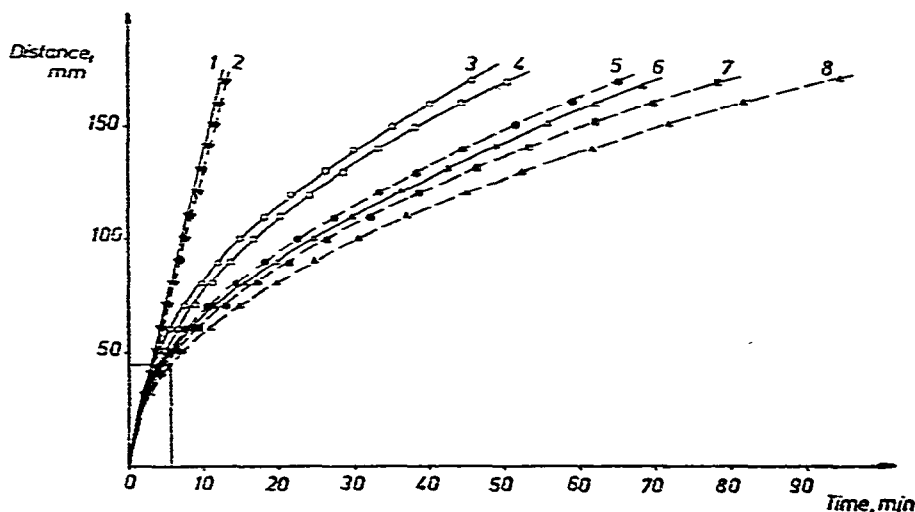


Fig. 2. Distance travelled by the solvent on normal and fine-particle silica gel layers versus time. Conditions as for Fig. 1. (1) PUM chamber, TLC plate; (2) PUM chamber, HPTLC plate; (3) N_n chamber, TLC plate; (4) UM chamber, TLC plate; (5) N_n chamber, HPTLC plate; (6) N_{ns} chamber, TLC plate; (7) UM chamber, HPTLC plate; (8) N_{ns} chamber, HPTLC plate.

particle silica gel plates, as a function of $R_F \times 100$ and distance of the start from "immersion depth" (s_0). It may be observed that the $R_F \times 100$ values of the three substances are always higher on HPTLC plates. It can be seen that the $R_F \times 100$

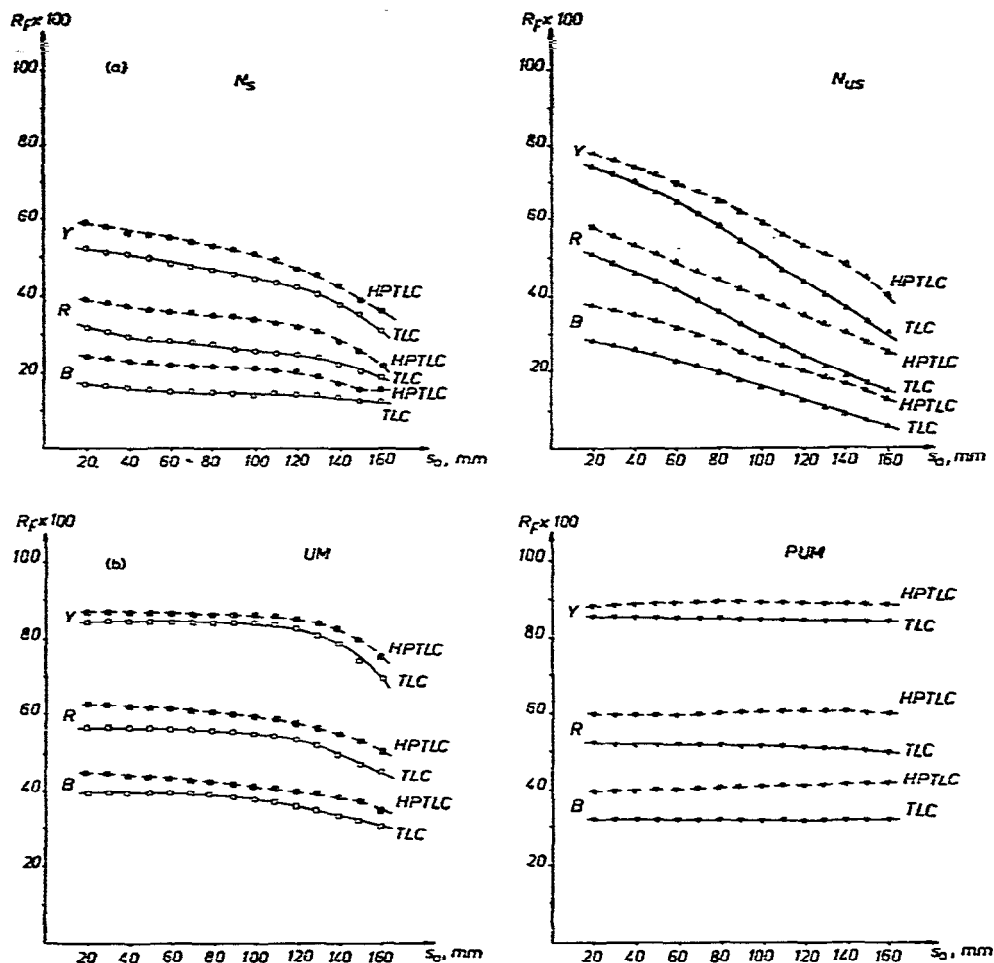


Fig. 3. Behaviour of the three dyes in different chambers using normal and fine-particle silica gel plates as a function of $R_F \times 100$ and distance of the start from immersion depth (s_0). Mobile phase, methylene chloride; sorbents, TLC aluminium sheets silica gel 60 F₂₅₄, 200 × 200 mm (Merck) and HPTLC aluminium sheets silica gel 60, 200 × 200 mm (Merck); temperature, 27°; distance migrated, 170 mm. Y = Butter Yellow; R = Sudan G; B = Indophenol.

values of the three dyes are practically constant in PUM chamber at different s_0 distances, whereas the R_F values decrease with increasing s_0 in N and UM chambers.

According to Guiochon and Siouffi³ the average plate height was calculated from

$$H = \frac{\sigma^2}{(L_F - s_0)R_F}$$

where L_F is the final distance of solvent front above the level of solvent in the reservoir or from solvent inlet, and s_0 is the distance of original (sample) spot over that level (the distance of the start from immersion depth).

It is apparent from Fig. 4, which gives the corresponding \bar{H} vs. L_F plots, that the efficiency of the fine-particle plates becomes poor for Butter Yellow with increasing L_F . The results are better with TLC plates. These data are similar to results of Guiochon and Siouffi³. It can be clearly seen that the relationship in the PUM chamber is close to linear on both plates. It is characteristic that the average plate height values on HPTLC plates are very small in the PUM chamber.

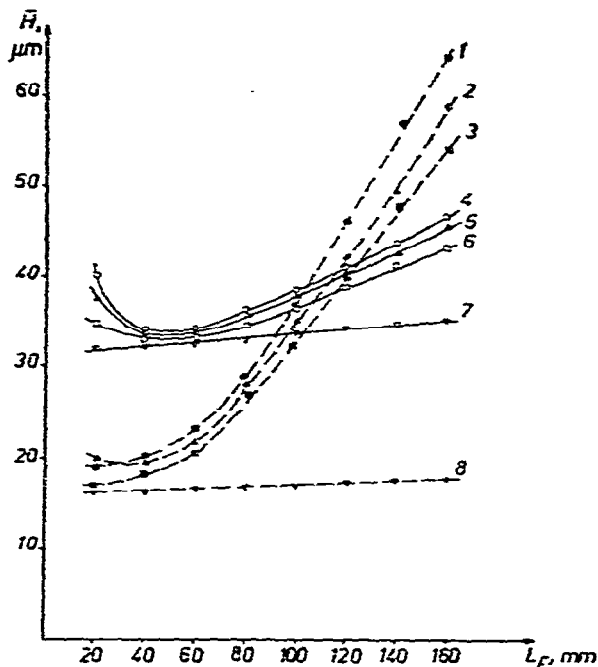


Fig. 4. Variation of the average plate height versus development length. Effect of different chambers and sorbent quality. Conditions as for Fig. 1, except $s_0 = 20$ mm. (1) N_2 chamber, HPTLC plate; (2) N_{20} chamber, HPTLC plate; (3) UM chamber, HPTLC plate; (4) N_2 chamber, TLC plate; (5) N_{20} chamber, TLC plate; (6) UM chamber, TLC plate; (7) PUM chamber, TLC plate; (8) PUM chamber, HPTLC plate.

Fig. 5 shows the variation of average plate height with s_0 . It can be seen that the values increase with increasing s_0 in N and UM chambers on both types of plate, but these values in the PUM chamber are in general smaller.

The flow-rate of the solvent can be increased only up to a defined limit, as at higher values (e.g., 80 ml/h) the spots will be deformed into protracted bands even on HPTLC plates. It should, however, be noted that flow-rates of 20 ml/h on fine-particle layers yield run times of only 10–12 min at 15–17 cm distances, and resolution values are very good (Table I).

CONCLUSIONS

The PUM chamber yields chromatograms with good resolution, particularly

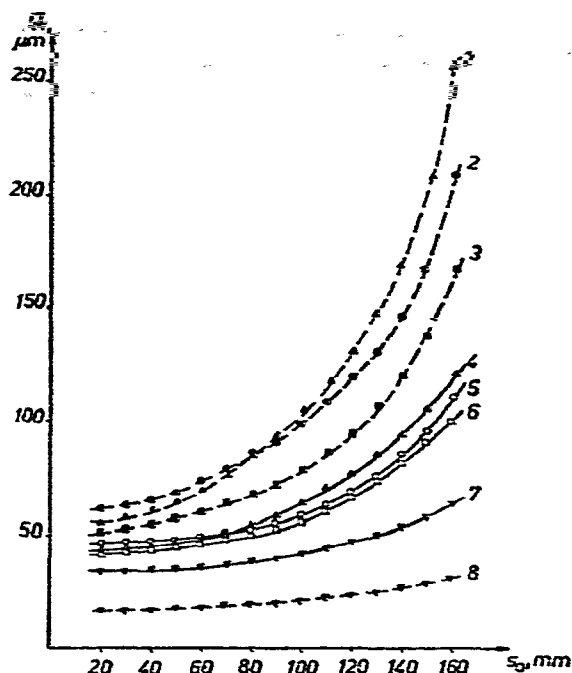


Fig. 5. Variation of the average plate height versus distance of the start from immersion depth (s_0). Conditions as for Fig. 1; $L_F = 170$ mm. (1) N_{2S} chamber, HPTLC plate; (2) N_2 chamber, HPTLC plate; (3) UM chamber, HPTLC plate; (4) N_{2S} chamber, TLC plate; (5) N_2 chamber, TLC plate; (6) UM chamber, TLC plate; (7) PUM chamber, TLC plate; (8) PUM chamber, HPTLC plate.

TABLE I

RESOLUTION VALUES OF THREE DYES ON NORMAL AND FINE-PARTICLE SILICA GEL 60 PLATES IN A LINEAR PUM CHAMBER

Mobile phase, methylene chloride; temperature, 20° ; sample, 0.6–0.6 μg of Test Dye Mixture I.

Distance migrated (mm)	Solvent flow-rate (ml/h)	R_s (Butter Yellow–Sudan G)		R_s (Sudan G–Indophenol)	
		TLC	HPTLC	TLC	HPTLC
150	20	9.65	12.41	6.90	8.50
	40	6.30	11.92	4.31	5.73
	60	6.22	8.91	3.92	4.12
	80	6.05	—	3.51	—
130	20	9.21	11.72	6.31	8.01
	40	6.55	11.51	4.05	5.52
	60	6.02	8.42	3.65	3.81
	80	5.63	—	3.00	—
110	20	8.43	10.84	5.57	6.90
	40	7.70	10.12	4.90	5.10
	60	6.61	6.81	3.72	3.18
	80	5.02	—	3.20	—
90	20	7.60	9.40	5.10	5.68
	40	6.80	9.10	4.21	4.48
	60	5.70	6.81	3.32	3.45
	80	5.40	—	3.04	—

suitable for quantitative evaluation, under the conditions of linear flow-rate of solvent and short run times, provided that fine-particle layers are used.

Working with multi-component solvents is much more complex. This problem will be dealt with in another paper.

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