Performance, Data and Results with Various Chromatographic Systems and Various Detection Patterns in High-Performance Thin-Layer Chromatography

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Summary

A knowledge of the relationships between particle size and layer thickness on the one hand and migration distance and plate height on the other has prompted the development of the pre-coated HPTLC plate for "nano-TLC". A separation performance which is very high with respect to time is achieved with a migration distance of 3–7 cm with a plate height of about 12 µm and several thousand theoretical plates, thanks to the possibility of simultaneous chromatography. In HPTLC quantitative evaluation, standardised chromatographic conditions are essential. In this context the complex influences of different types of chamber and solvents on linear and circular chromatography are described. Various methods of conditioning the adsorbent by immersion impregnation, as well as detection procedures are discussed. The scope for varying TLC procedures and the advantages of TLC in general are listed.

Development of the pre-coated HPTLC plate

During the past three years important relationships have been established between primary and secondary features of adsorbent layers, i.e., between pore system and activity on the one hand and particle size and particle size distribution on the other, and their chromatographic properties. Fig. 1 can be considered as a preliminary result of our work. It shows a circular chromatogram with about 50 micro spots of 20 nl each in an eccentric application pattern. The chromatogram was completed within about 10 minutes, accurate dose application and actual chromatography included. A very high degree of precision was obtained thanks to the quality of the layer, which was confirmed by means of scanning electron micrography to consist of an extremely dense packing of particles of practically uniform size and with a smooth, homogeneous surface. Fig. 2 compares the surface homogeneity of the conventional pre-coated silica gel TLC plate with that of its high-performance counterpart. The surface patterns were obtained from photometric measurements with the layers inclined at an angle of 10° and from microscopic surface photographs.

In a very broad study programme adsorbent layers consisting of silica gels of different particle size and different layer thickness, with otherwise equal conditions, e.g., in terms of silica gel-type, binder and indicator additions, suspension and coating procedure, were applied. Chromatography was then performed, the same chamber type, presaturation of layer, temperature and coating procedure being used, whereas the migration distances of the solvents were different and three different sample sizes were used in a lipophilic system and, partly also, in a hydrophilic system. The evaluation of three spots in triplicate, with four different particle size ranges, three different layer thicknesses, three different sample sizes, and ten migration distances, yielded a total of 3240 individual data. The reflectance measurements were carried out on a Zeiss chromatogram spectrophotometer PMQ II, the analogue output being linked to a process control computer IBM 1800.

Parameters determined:

t_f	s	Migration time of solvent from starting line to front
t_{f_0}	s	Migration time of solvent from immersion level to starting line
t	s	Development time of chromatogram
z_f	mm	Migration distance of solvent from starting line to front
z_{f_0}	mm	Migration distance of solvent from immersion level to starting line
z_x	mm	Migration distance of substance
κ	mm^2/s	Velocity coefficient
hR_f		Percentage of substance in mobile phase
w_x	mm	Base width of substance peak
H	µm	Plate height
R_s		Resolution

Parameters determined occasionally:

k		Partition factor
a		Relative retention, selectivity
N′		Effective plate number
N′/s	s^{-1}	Effective plate number/sec

Figs. 3 and 4 show H-z_f curves. The migration distances z_f in mm are plotted on the abscissa, the plate heights H in µm are plotted on the ordinate. It has proved of value to relate the individual plate heights to a mean hR_f value of 50. In this way all spots within an hR_f range from 20 to 80

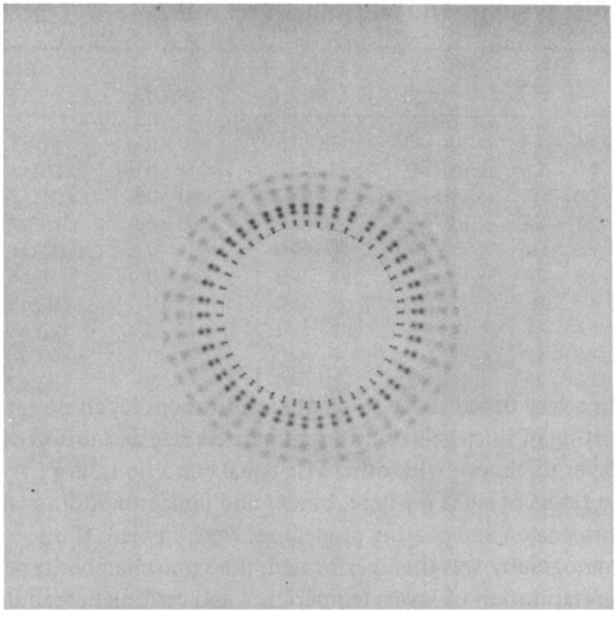

Fig. 1

● Circular chromatogram

Eccentric application of 20 nl = 20 ng each of lipophilic dyes using a 1 µl Hamilton syringe with micrometer; z_{f_0} = 15 mm; z_f = 20 mm.

are covered. In Fig. 3 the four horizontal rows are related to different mean particle sizes of silica gel, with the coarser grades above and the finer grades below. The three vertical rows are related to different layer thicknesses covering the range from about 300 to 100 µm, with the larger numbers to the left and the smaller numbers to the right. Carefully classified silica gels were used in each case. The three curves of each individual diagram are related, from above to below, to three different application volumes, i.e., 2 µl, 0.75 µl and 0.1 µl, corresponding to 2000 ng, 750 ng and 100 ng of individual substance.

Within the horizontal rows of different particle sizes an overall decrease of plate heights from row 4 to row 3 and from row 3 to row 2 can be recognised, whereas a strong increase is noted from row 2 to row 1. Within the vertical rows of different layer thicknesses, row I with the smallest layer thickness always shows larger, i.e. more unfavourable, plate heights as compared to those of rows II and III, which show hardly any difference. The optimum plate height values can be read from the path of the individual H-z_f curves.

The path of a curve is influenced by the particle size range of the silica gel used. In the uppermost horizontal row 4 the optimum plate height value has not yet been reached

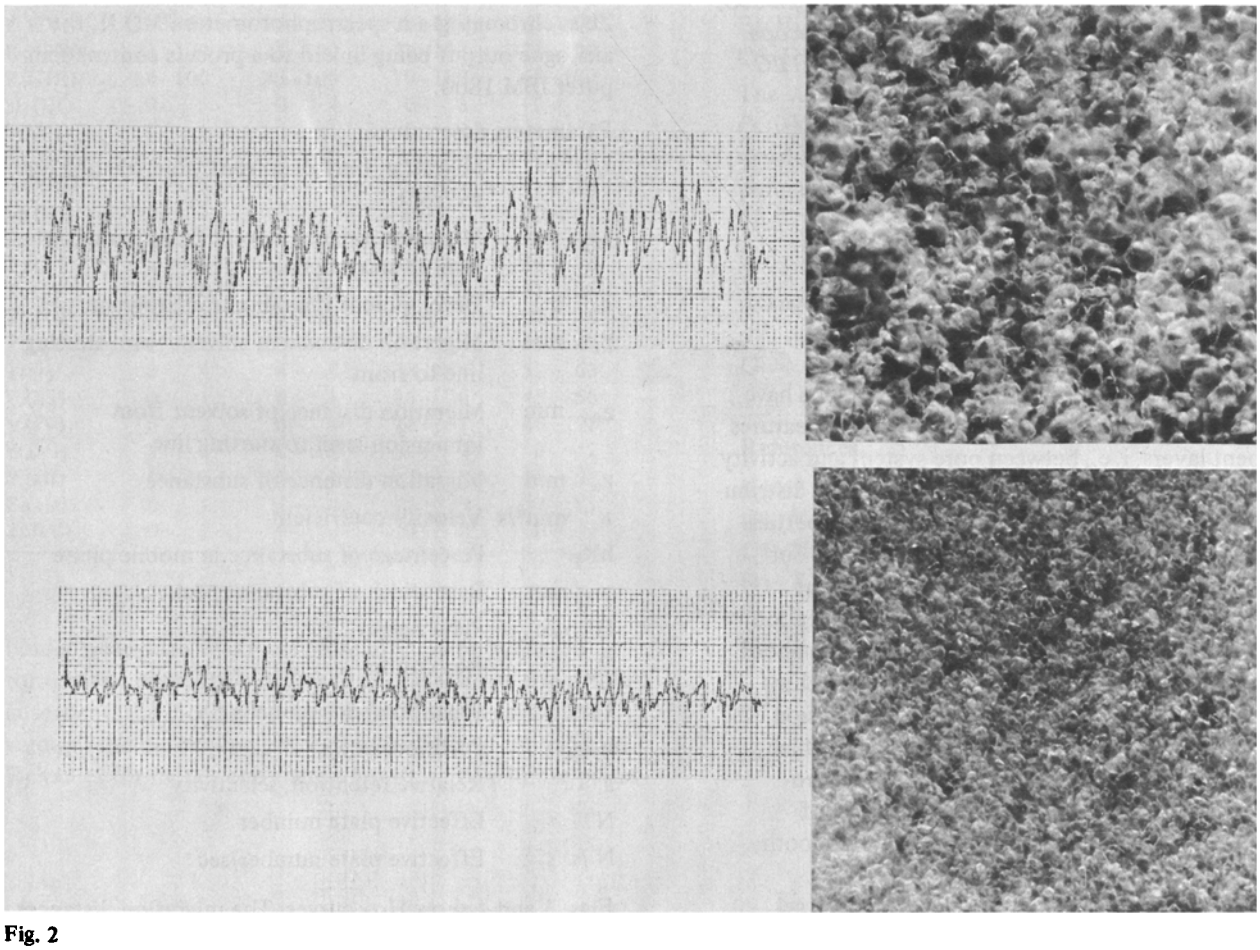

Fig. 2

● Photometric surface measurements and microscopic surface photographs of pre-coated TLC plates.

Top: pre-coated TLC plate silica gel 60 F_{254}; bottom: pre-coated HPTLC plate silica gel 60 F_{254} for nano-TLC; left: slit 2.5 mm × 2.5 mm, magnification 1 : 24, angle of incidence 10°; right: layer treated with ceres violet, magnification 1 : 200, incident light.

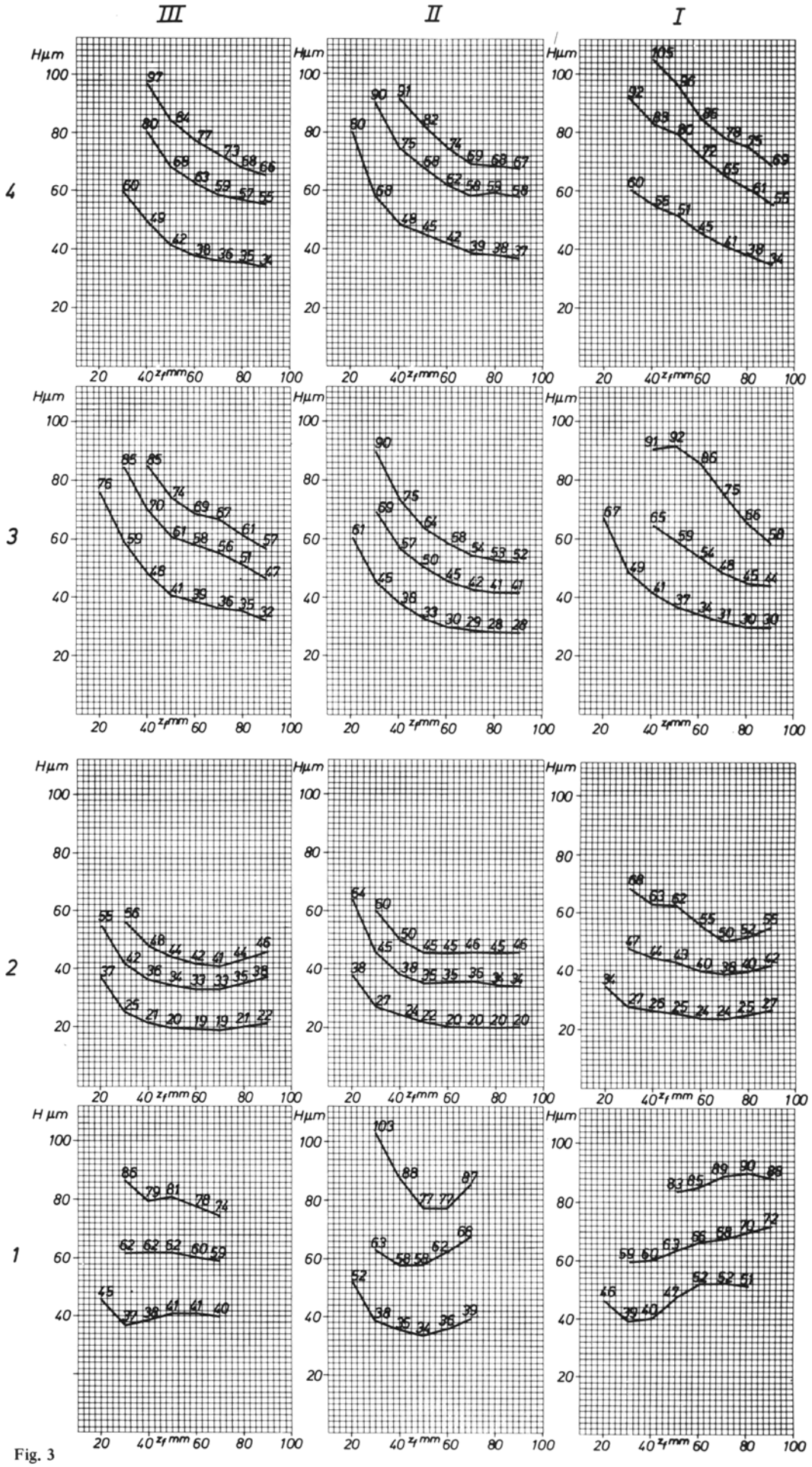

Fig. 3

- $H - z_f$ curves of pre-coated TLC plates (extended experimental studies).

  Abscissa = migration distance z_f (10 – 100 mm); ordinate = plate height H (μm); horizontal rows 4 – 1 = particle sizes from coarser to finer; vertical rows III – I = layer thicknesses from larger to smaller; application amounts: upper curve 2 μl = 2000 ng each lipophilic dyes, medium curve 0.75 μl = 750 ng each, lower curve 0.1 μl = 100 ng each.

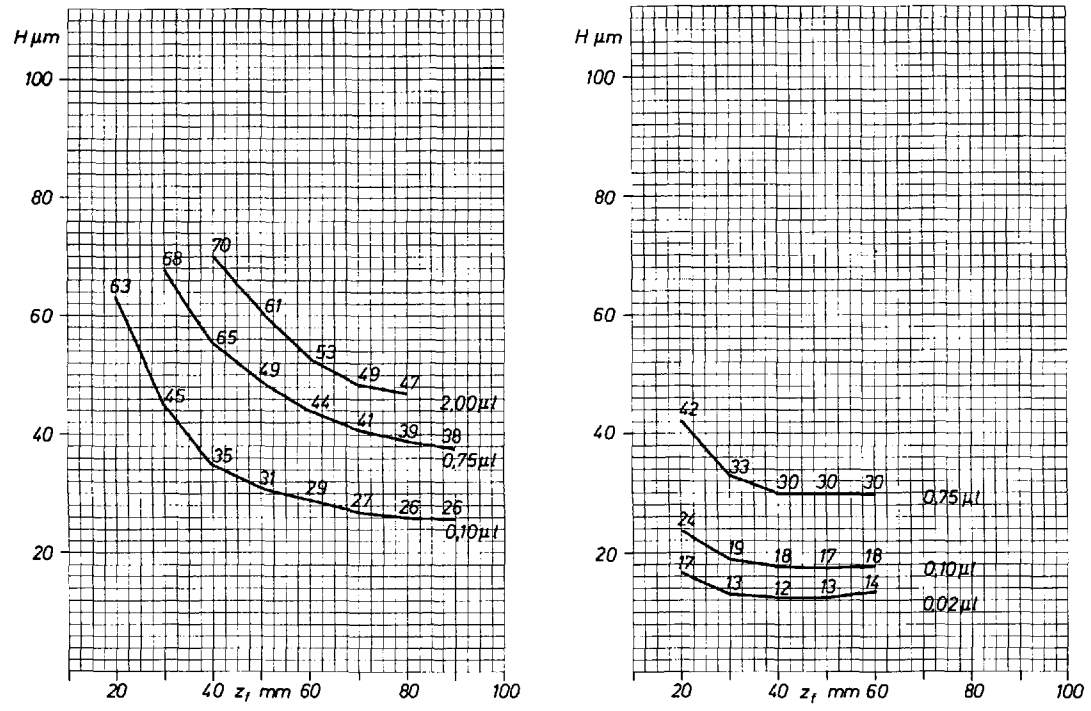

Fig. 4

- $H - z_f$ curves of pre-coated TLC plates (production setting).

Left: pre-coated TLC plate silica gel 60 F_{254}; right: pre-coated HPTLC plate silica gel 60 F_{254} for nano-TLC; abscissa = migration distance z_f (10 – 100 mm); ordinate = plate height H (µm); application amounts of lipophilic dyes: left 2.0 µl (2000 ng each), 0.75 µl (750 ng each), 0.1 µl (100 ng each); right 0.75 µl (750 ng each), 0.1 µl (100 ng each), 0.02 µl (20 ng each).

at a migration distance of 100 mm. In row 3 the optimum value is found at about 100 mm, whereas in row 2 it is found at a distinctly lower level, at about 50 mm; it is to be noted here that the curves rise only very gradually as the migration distances become larger. In row 4, associated with a silica gel of very fine particle size, the optimum value is found at even lower migration distances; it should be noted that in this case there are some very sharp rises with the larger migration distances. From these patterns it can be concluded that each particle size range of an adsorbent can be associated with an optimum migration distance and thus with an optimum plate height. Diagramms 3/II and 2/II are grossly related to the particle size ranges of conventional pre-coated TLC plates and of the HPTLC pre-coated plates. While the results obtained in the coating studies discussed so far were derived from extended experimental studies the results shown in Fig. 4 are derived from a production setting. The results obtained in this latter case are clearly superior to the extended experimental ones. This can be explained by the superior coating procedures achieving higher packing density. With the pre-coated TLC plate (left diagram) optimum plate heights of about 30 µm are obtained. With the HPTLC pre-coated plate (right diagram) plate heights of 12 µm are obtained with even smaller application volumes. The application of minute amounts down to 5 nl is very easy to perform if a Hamilton syringe (1 µl) is used in connection with a micrometer. In this way about ten spots can be applied per minute with a high degree of accuracy. Whereas conventional thin-layer chromatography called for applications of a few microliters, the volumes used in high-performance thin-layer chromatography must be distinctly lower in the nanolitre range.

Chromatographic performance of the pre-coated HPTLC plate

Fig. 5 shows the number of available effective plates N′ with different migration distances z_f as a function of the plate height H, based on a maximum hR_f value of 80, which prevails in chromatography using a normal chamber (N-chamber). Plate heights between 10 and 15 µm, which can be obtained in HPTLC, give several thousands of effective plates depending on the migration distance.

Fig. 6 gives a tabulated survey of the number of complete separations of neighbouring pairs of substances, again based on a maximum hR_f value of 80, with different migration distances z_f and different plate heights H, and based on 4σ separations, corresponding to a resolution $R_s = 1$.

The large number of complete separations obtained even with short migration distances within the plate height range from 10 to 15 µm, i.e., the range of interest in this connection, is remarkable. Since the resolution R_s is inversely proportional to $2\sqrt{H}$, a decline in plate height from the conventional pre-coated TLC plate (about 30 µm) to the pre-coated HPTLC plate (about 12 µm) means an improvement in resolution of about 60 %, i.e., a remarkable gain in performance.

plate height H (hR_f 50) µm	different migration distances z_f 25 mm = 20 mm (hR_f=80)	50 mm = 40 mm (hR_f=80)	75 mm = 60 mm (hR_f=80)	100 mm = 80 mm (hR_f=80)
50	400	800	1 200	1 600
40	500	1 000	1 500	2 000
30	667	1 333	2 000	2 667
25	800	1 600	2 400	3 200
20	1 000	2 000	3 000	4 000
15	1 333	2 667	4 000	5 333
10	2 000	4 000	6 000	8 000
5	4 000	8 000	12 000	16 000

Fig. 5

- Number of effective plates N′ depending on plate height and migration distance.
  Basis: maximum hR_f value = 80.

plate heigth H (hR_f 50) µm	different migration distances z_f 25 mm = 20 mm(hR_f=80)	50 mm = 40 mm(hR_f=80)	75 mm = 60 mm(hR_f=80)	100 mm = 80 mm(hR_f=80)
50	6,3	8,9	10,8	12,6
40	7,1	10,0	12,2	14,1
30	8,2	11,5	14,1	16,3
25	8,9	12,6	15,5	17,9
20	10,0	14,1	17,3	20,0
15	11,5	16,3	20,0	23,1
10	14,1	20,0	24,5	28,3
5	20,0	28,3	34,6	40,0

Fig. 6

- Number of complete separations of neighbouring pairs of substances (4 σ separations corresponding to R_S = 1), depending on plate height and migration distance.
  Basis: maximum hR_f value = 80.

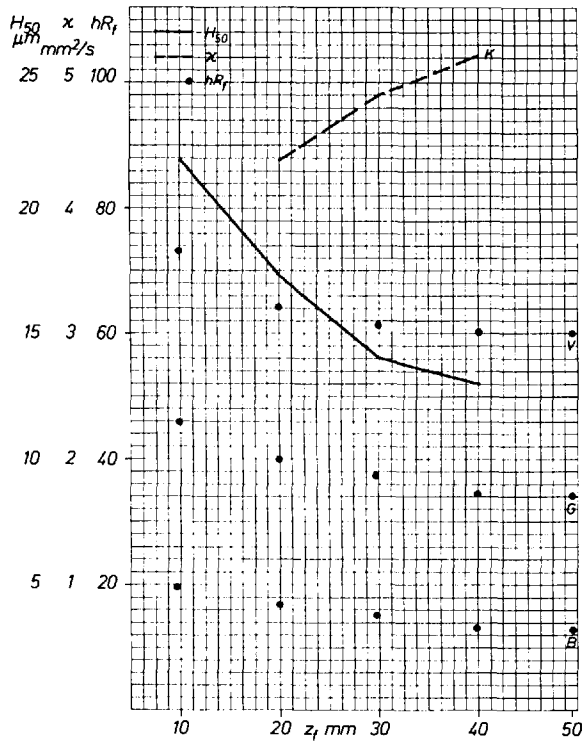

Fig. 7

- Chromatographic characteristics (hR_f values, H values and κ values) depending on migration distance.
  N-chamber/saturation; benzene; 20 nl = 20 ng each lipophilic dyes; 8 measuring points each.

Fig. 7 shows the changes in the hR_f values, plate height H and velocity coefficient κ for a migration distance z_f with linear development in an N-chamber with saturation of the atmosphere. As the migration distance increases, the hR_f values of the three dyes violet (V), green (G) and blue (B) decline, due to an increasing uptake of solvent via the vapour phase. For the same reason the velocity coefficient κ is increased because less solvent is required via the capillary flow for pore filling with larger migration distances z_f and correspondingly longer rest times. The diagrams in Figs. 3 and 4 have already shown a decline in plate height H with increasing migration distances z_f in the 10–50 mm range.

The use of the more lipophilic solvents as a rule results in a decline of R_f values whereas an increase is obtained from the more hydrophilic solvents. In selecting solvents for routine work, solvents with higher κ values, i. e., shorter migration times, will be preferred. Butanol and propanol, too, may be used to advantage for special TLC separations in spite of their unfavourably low κ values.

Fig. 8 contains a tabulated presentation of linear ascending chromatograms with different κ values of the solvents used and different migration distances z_f, allowance having been made for a 5 mm distance z_{f_0} between the immersion level of the solvent and the starting line. Since the law of flow for TLC involves the square of the migration distance, the development times are prolonged considerably with larger migration distances, particularly in the case of solvents with low κ values.

So, the separation efficiency expressed as number of effective plates N′/sec, is significantly higher with small migration distances z_f and with layers of low plate height than with larger migration distances and more unfavourable plate heights. These relationships are shown in tabulated form in Fig. 9, based on a maximum hR_f value of 80, a κ value of the solvent of 10 $mm^2\ s^{-1}$ as well as parallel development of ten chromatograms per plate. Of course the resolution and separation number are increased with a larger migration distance, in spite of a more unfavourable separation efficiency expressed as number of effective plates N′/sec.

velocity coefficient	different migration distances z_f				$t\ (sec) = \frac{(z_f + 5)^2}{\kappa}$
mm^2/sec	25 mm	50 mm	75 mm	100 mm	
2	7,5	25,2	53,3	91,9	
5	3,0	10,1	21,3	36,8	
10	1,5	5,1	10,7	18,4	
15	1,0	3,4	7,1	12,3	
20	0,8	2,5	5,3	9,2	
25	0,6	2,0	4,3	7,4	
30	0,5	1,7	3,6	6,1	

Fig. 8

- Development times t (min) of chromatograms depending on velocity coefficient and migration distance.

plate height H (hR_f 50) µm	different migration distances 25 mm = 20 mm (hR_f=80)	50 mm = 40 mm (hR_f=80)	75 mm = 60 mm (hR_f=80)	100 mm = 80 mm (hR_f=80)
50	44	26	19	15
40	56	33	23	18
30	74	44	31	24
25	89	53	38	29
20	111	66	47	36
15	148	88	63	48
10	222	132	94	73
5	444	264	188	145

Fig. 9

- Separation efficiency per chromatogram, expressed as number of effective plates N'/sec, depending on plate height and migration distance.
  Basis: maximum $hR_f = 80$.

Influence of type of chamber

Figs. 10 and 11 show that chromatographic characteristics are influenced by the type of chamber used as well as by the activity of the adsorbent. The conditions prevailing in a normal chamber with saturation of the atmosphere as well as in a narrow chamber (S-chamber) (1–2 mm spaced) and the conditions prevailing in a Vario-KS-chamber with different relative humidities of the adsorbent (between 0 % and 80 %) are plotted on the left and right sections of the abscissae. The hR_f values and the κ values (Fig. 10) and the values for plate heights and resolutions of neighbouring pairs of substance (Fig. 11) are plotted on the associated ordinates. As compared to the N-chamber with a saturated atmosphere, in the S-chamber the hR_f values are increased considerably, especially in the higher range, due to the fact that there was hardly any presaturation of the layer via the vapour phase. For the same reason the κ values are lower, i. e., the migration times are somewhat prolonged. The increase

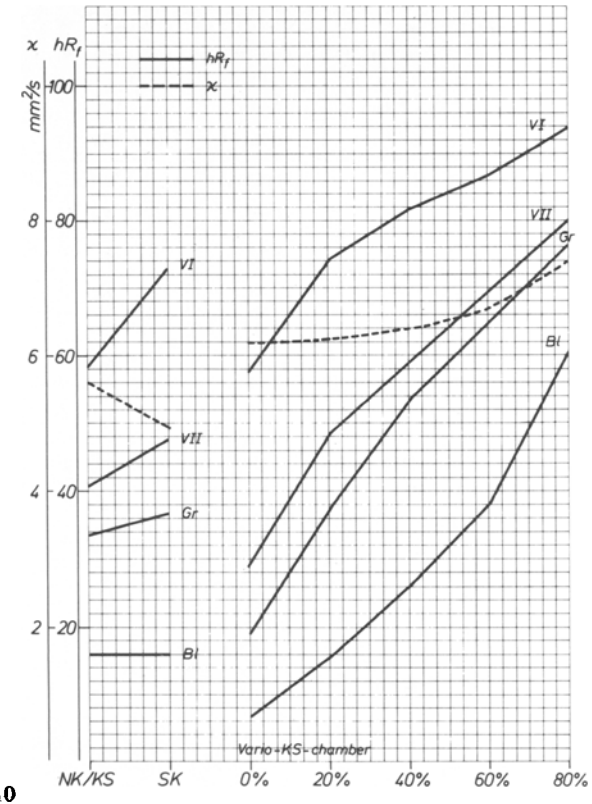

Fig. 10

- Chromatographic characteristics (hR_f values, κ values) for various types of chamber (N-chamber/saturation, narrow chamber) and with different relative humidities (Vario-KS-chamber).
  z_f = 50 mm; benzene; 30 nl = 30 ng each lipophilic dyes.

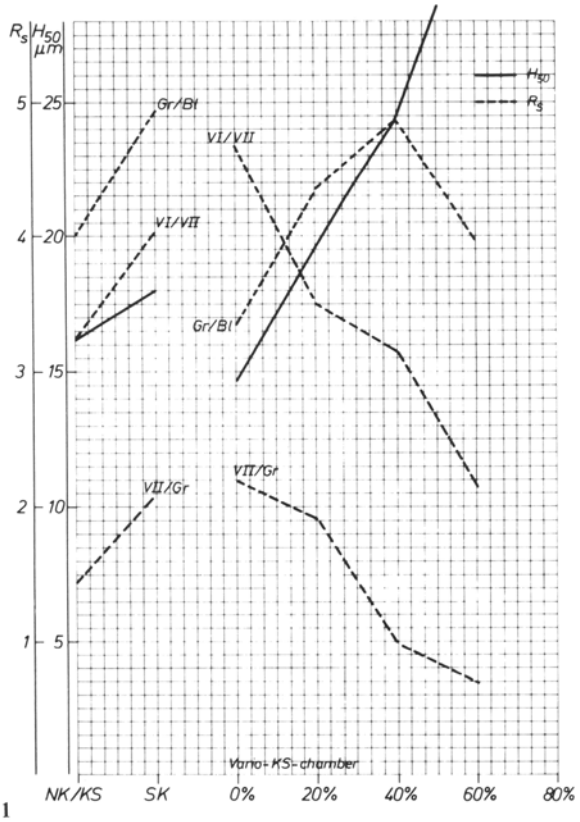

Fig. 11

- Chromatographic characteristics (H values, R_s values) for various types of chamber (N-chamber/saturation, narrow chamber) and with various relative humidities (Vario-KS-chamber).
  z_f = 50 mm; benzene; 30 nl = 30 ng each lipophilic dyes.

in R_f values with higher humidity figures in the Vario-KS-chamber is produced by the selectively higher inactivation of the adsorbent. The slight increase in plate height in the narrow chamber as compared to the normal chamber with saturated atmosphere cannot be explained satisfactorily. There might be a connection between this behaviour and the somewhat longer migration time. The strong increase in plate height with increasing relative humidity in the Vario-KS-chamber is explained by the increasing deactivation of the adsorbent (hydration of the silanol groups). The improvement in substance-pair resolutions in the narrow chamber as compared to the saturated normal chamber is attributed primarily to the larger differences in hR_f values. On the other hand, the deterioration in resolution with adsorbents with higher humidities in the Vario-KS-chamber is attributable to the increase in plate height.

It was found that the commercially available narrow chambers, with a 1–2 mm spacing between layer and counterplate secured by a cardboard or metal frame, still enable presaturation of the layer via the vapour phase, however small this presaturation may be. For this reason narrow chambers with variable spacings down to 0.1 mm devised in our own laboratory were used for the subsequent experiments (Fig. 12). Small discs punched from an aluminium or plastic foil of adequate strength were placed on four marginal sites of the layer of a 10 × 10 cm pre-coated HPTLC plate with spotted or streaked applications over a distance of 15 mm. A 9 cm × 10 cm counterplate made of glass was placed on top, flush with three sides and fixed with four lateral clamps to the coated plate. The narrow chamber constructed in this manner was placed vertically into a normal chamber for larger plate sizes, i.e., 20 × 20 cm or 10 × 20 cm, filled with solvent to a height of about 5 mm, the solvent being freely taken up via the free coating strip. The extremely small spacing between coating and counterplate of 0.1 mm prevents presaturation of the layer via the vapour phase and enables the determination of real R_f-values. The use of a larger chamber as a solvent reservoir for housing the smaller narrow chamber offers additional protection against temperature and air draught fluctuations during chromatography, which is necessary because the migration pattern and thus the R_f-values must be held constant over the entire width of the layer.

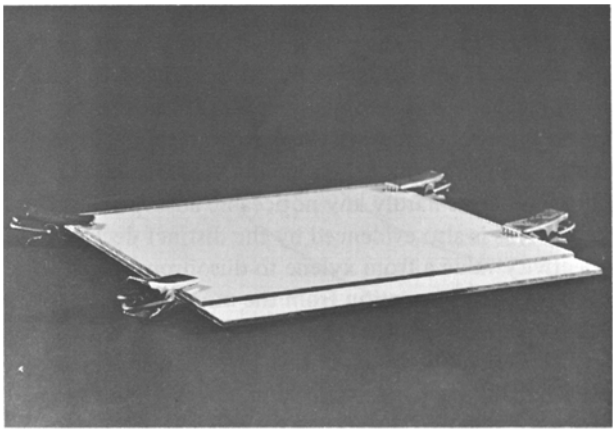

Fig. 12

- Narrow chamber with variable spacing down to 0.1 mm consisting of pre-coated HPTLC plate 10 cm × 10 cm, 4 spacer discs, glass or silica gel counterplate 9 cm × 10 cm, 4 clips.

The changes in some of the chromatographic characteristics, which are attributable solely to the type of chamber used, are shown in Fig. 13. There are practically no differences between the narrow chambers with 0.1 mm and 0.2 mm spacing as regards the R_f-values, plate heights and κ-values. While with the normal chamber with chamber saturation the R_f-values and κ-values differ considerably from those of the < 0.2 mm narrow chamber, there is a good agreement as to these values in the case of the normal chamber without saturation and the < 0.2 mm narrow chamber. A slight decline in R_f-values, up to about 5 %, is observed in the upper range. However, the distinct improvement in plate height in the normal chamber as compared to the narrow chamber is remarkable. Transverse diffusion is obviously slightly increased with the latter. The extremely small spacing always causes the solvent to be retained for some time at the surface of the layer up to the height of the solvent front, from where it cannot evaporate. The possibility of evaporation in the case of the normal chamber results in more favourable plate heights, although in the case of the normal chamber with chamber saturation the absolute plate height values deteriorate on account of the lower R_f-values.

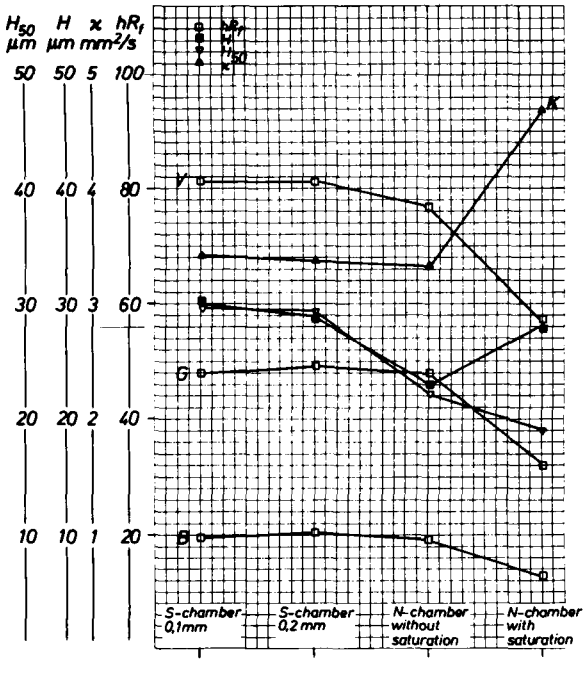

Fig. 13

- Chromatographic characteristics (hR_f values, κ values, H values) with various types of chamber (S-chamber 0.1 mm, S-chamber 0.2 mm, N-chamber without saturation, N-chamber with saturation),

  z_f = 30 mm; benzene; 100 nl = 100 ng each lipophilic dyes.

Further interesting information on the influence of evaporation effects on chromatography is obtained from experiments in which the counterplate in a < 1 mm narrow chamber is not made of glass but consists of an activated silica gel layer on a glass plate. In this setting too the solvent flow is guided by the capillary forces to the upper section of the plate. However, a larger amount of solvent migrates through the chromatographic layer because of the simultaneous rapid evaporation of the solvent from the surface of the chromatographic layer directly to the opposite active silica gel layer.

The results achieved with this setting are presented in Fig. 14, spacings of 200 μm and 600 μm having been chosen. The abscissa shows the chromatographic data of the normal chamber without chamber saturation (middle), those of the 0.2 mm narrow chamber (left) and those of the 0.8 mm narrow chamber (right), a glass or activated silica gel on glass counterplate being used. As expected,

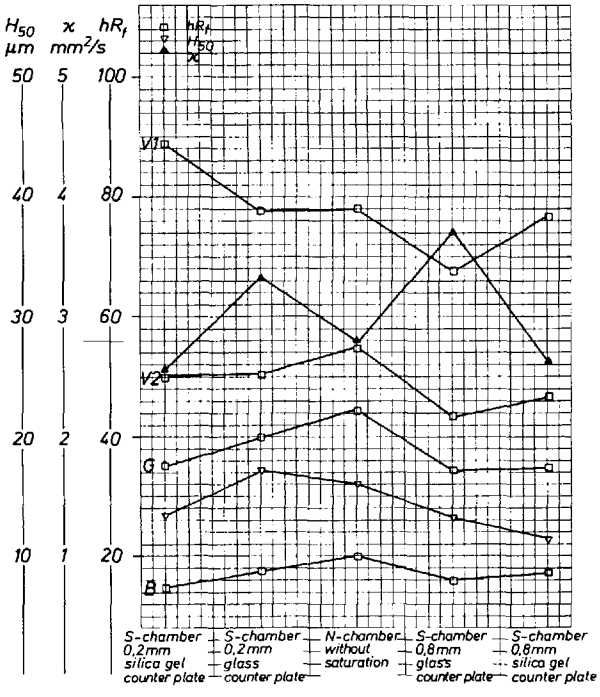

Fig. 14

- Chromatographic characteristics (hR_f values, κ values, H values) with various types of chamber (N-chamber without saturation, S-chambers 0.2 mm and 0.8 mm with glass and silica gel counterplate, respectively),
  $z_f = 35$ mm; benzene; 30 nl = 30 ng each lipophilic dyes.

differences were found with the κ-values. In the silica gel counterplate chamber smaller κ-values were measured than in the glass counterplate chamber. This is accounted for by a simulated prolongation of the migration time caused by the higher solvent throughput. It is possible to calculate the solvent take-up of the silica gel counterplate from the difference in the κ-values of the two types of chamber. The relatively low κ-value of the normal chamber without chamber saturation suggests that the evaporation effect plays a considerable role in this type of chamber too. In the case of the chamber with the glass counterplate the R_f-values are lower with the 800 μm spacing than with the 200 μm spacing due to presaturation in the additional chamber. In the case of chambers with 800 μm spacing, somewhat higher R_f-values were measured with the silica gel counterplate than with the glass counterplate, which is attributable to the greater solvent flow. In the case of chambers with 200 μm spacing the R_f-values are higher from the beginning. Here a further increase in the R_f-value is found only in the upper R_f range with the silica gel counterplate as compared to the glass counterplate. No significant differences are found in the lower R_f range. The highest relative retention values are found with the 0.2 mm narrow chamber with the silica gel counterplate, while the lowest values are associated with the 0.8 mm narrow chamber with glass counterplate. The plate heights are more favourable with the two chambers with silica gel counterplate as compared to those with the glass counterplate.

These experiments show the great variability of chromatographic systems relying on layers. In spite of identical coatings and identical solvents considerably different chromatographic results are obtained solely due to different types of chamber. Optimal conditions should be aimed at, starting from the chromatographic system. In quantitative evaluations this is a prerequisite.

Besides the velocity coefficient, other characteristics such as density, boiling point, vapour pressure or evaporation number should also be considered in the selection of a solvent, or, even more so, a solvent mixture. According to our own findings, the surface tension of a solvent does not play any role in chromatography. In the presence of an adsorbent the value γ is obviously altered to a large extent. However, the viscosity of a solvent is a very important factor which has a distinct influence on the κ value and thus on the migration time. The decline in viscosity at higher temperatures has a favourable influence on the κ values.

Fig. 15 shows the relationships between a number of chromatographic characteristics and the solvent used in each case. In accordance with the polarity sequence [1], the uptake of the substance by the adsorbent declines from xylene to diisopropylether, which leads to an increase in the R_f values. In the case of diisopropylether, separation of the three dyes violet (V), green (G) and blue (B), yields hardly any noticeable adsorption differences. This is also evidenced by the distinct decline in the selectivity value a from xylene to diisopropylether. The decline in the adsorption from the more lipophilic to the less lipophilic solvents is also shown by an increased diffusion of spots, which in turn results in an increase in plate height H and a deterioration of the separation quality.

In order to obtain consistent hR_f values over the entire width of the layer, a minimum migration distance is required in spite of a high degree of accuracy with regard to constant chromatography conditions in terms of

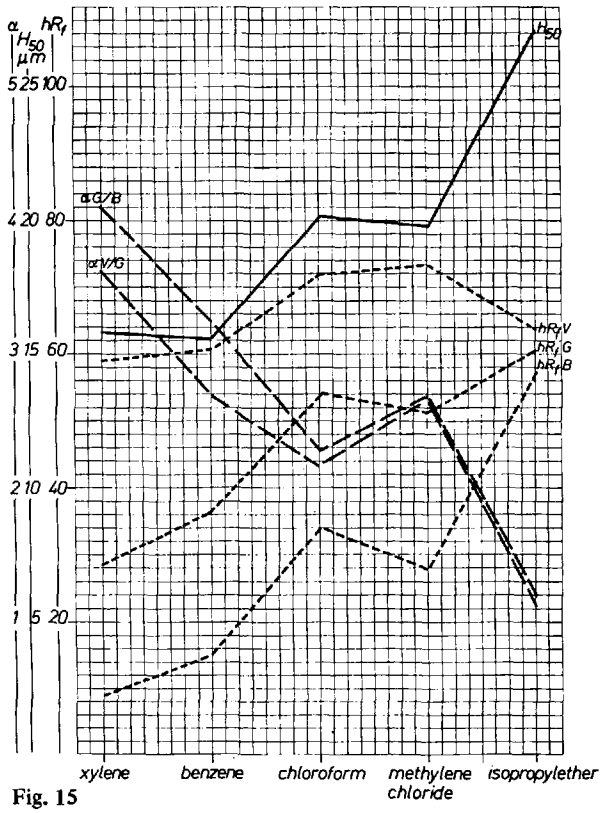

Fig. 15

- Chromatographic characteristics (hR_f values, H values, α values) depending on solvent used.

  N-chamber/saturation; z_f = 50 mm; 50 nl = 50 ng each lipophilic dyes.

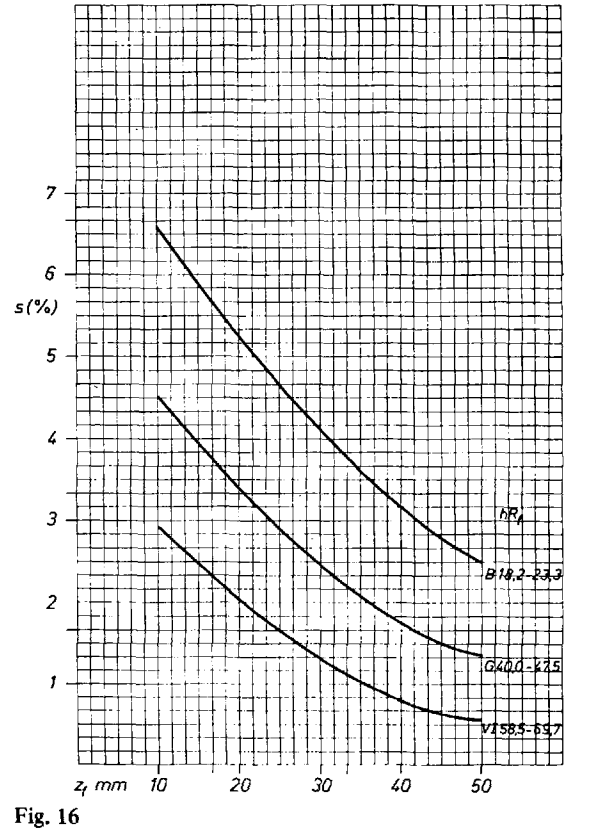

Fig. 16

- Standard deviations of hR_f values depending on migration distance.

  Abscissa = migration distance z_f (mm); ordinate = standard deviation s (%); N-chamber/saturation; benzene; 20 nl = 20 ng each lipophilic dyes; eight applications at 10 mm intervals.

development chamber, pre-saturation and temperature (Fig. 16). So, for instance, a standard deviation of hR_f values below 1 % is obtained for the dye VI with the highest R_f value between 0.6 and 0.7 at a migration distance z_f of the solvent of about 35 mm, which means a migration distance z_x of the substance of about 20 mm.

The two other dyes, G and B, with smaller absolute migration distances, show correspondingly more unfavaourable standard deviations in their hR_f values. Certain errors occurring during application of the substances at the same height (not dosing errors) or during initial wetting of the layer are corrected gradually during the process of solvent flow.

The relations between the peak areas of the individual substances, too, are subject to change as the migration distances z_f change (Fig. 17). As a consequence, absolutely consistent R_f values must be ensured for the entire plate if an internal standard is used for quantitative determination. The good harmony of R_f values is shown in Figs. 18 and 19. In this case amounts of 30 nl of solution each were spotted by means of a Hamilton syringe and 1 µl of solution was applied in a line measuring 80 mm by means of a Hamilton syringe and an automatic applicator. Development was carried out in a normal chamber with saturation of the atmosphere under standard conditions.

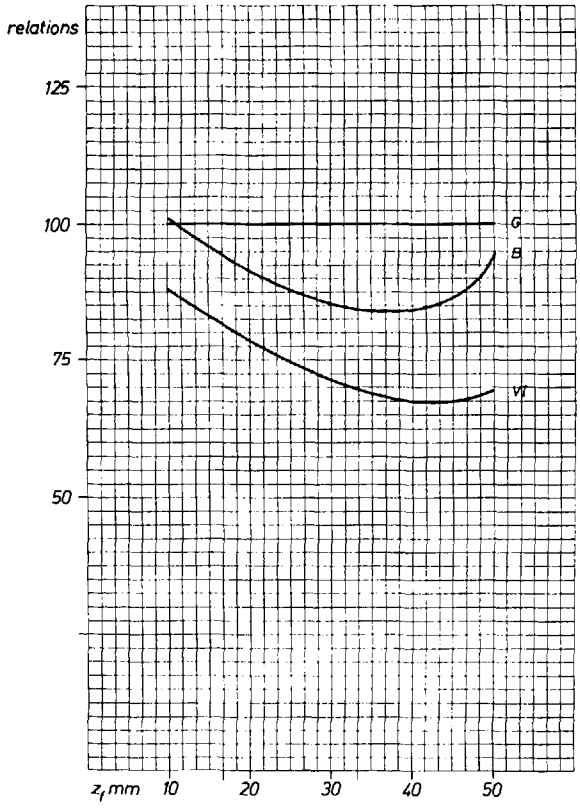

Fig. 17

- Relations between peak areas (mVsec) of three different substances depending on migration distance.

  Abscissa = migration distance z_f (mm); ordinate = relations of peak areas related to dye G (100) with medium R_f values; N-chamber/saturation; benzene; 20 nl = 20 ng lipophilic dyes ceres violet BRN (V), ceres green (G), solvent blue (B); reflectance measurements at 586 nm; eight measuring points each.

Comparison between linear and circular chromatography

In the diagrams that follow linear chromatography and circular chromatography are compared for some chromatographic characteristics on the basis of different migration distances z_f of the solvent. Development is carried out in the ascending way in a saturated N-chamber or in a Petri dish with solvent transfer to the inverted layer via a felt wick of 2 mm diameter, that means in any case in chambers allowing partial pre-loading of the layer via the vapour phase of the solvent. In Fig. 20 the resolutions R_s of two dye pairs each in a lipophilic chromatographic system with linear and circular chromatography with centric application are compared. In both the migration distance z_f is measured from the contact point of the solvent depot with the adsorbent layer to the solvent front.

In the case of the separation of the substance-pair with higher R_f values, i.e., the violet and the green dye, the resolution R_s between 20 and 50 mm shows a very large increase for linear chromatography, while a moderate increase is shown for circular chromatography. Only at a migration distance of 20 mm is an equivalent separation efficiency obtained for the two methods. At a 50 mm migration distance the resolution ratio is 5.1 : 3.4 in favour of linear chromatography, which means that separation is improved by 50 %. Things are somewhat different when comparing the separation efficiency of substance-pairs with lower R_f values, i.e., the green and the blue dyes. Here a large increase in resolution with increasing migration distance is found in both chromatographic systems. With a migration distance of 20 mm, the resolution is 24 % more favourable in circular chromatography than in linear chromatography, but the figure is reduced to 3 % with a migration distance of 50 mm. These results confirm the well-known fact that circular chromatography favours the separation of substances with lower R_f values. The very much lower κ value for circular chromatography as compared with linear chromatography is notable. It amounts to no more than about one-third in the case of partially pre-loaded layers, which means that in circular chromatography development times must be about three times longer than in linear chromatography when the migration distance is the same.

In Fig. 21 a comparison is made of some chromatographic data for non-presaturated chamber systems for linear and circular chromatography, with the latter not only providing for a solvent flow from the centre to the periphery, as usual, but also from the periphery to the centre. Solvent

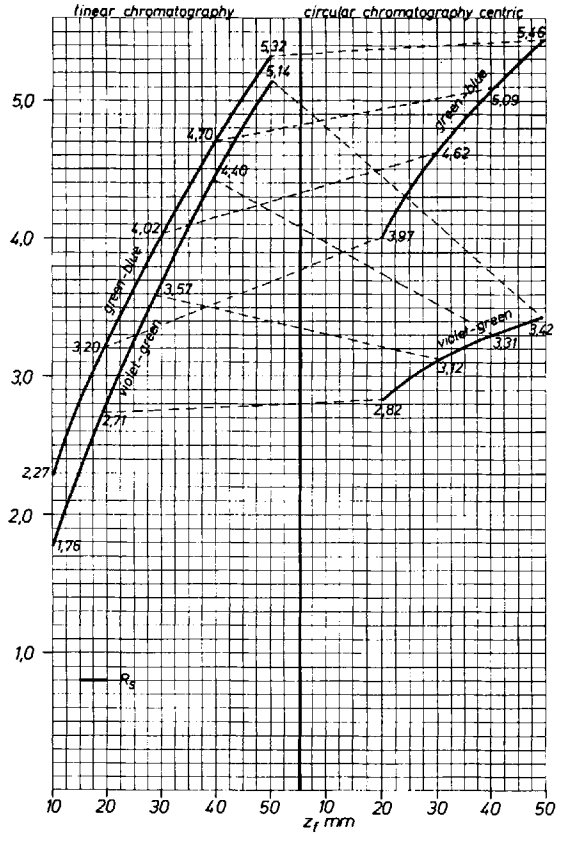

Fig. 20

- Comparison of chromatographic characteristics (R_s values) between linear and circular chromatography (centric application), depending on migration distance.

  Abscissa = migration distance z_f (10 – 50 mm); ordinate = resolution R_s, linear chromatography: N-chamber/saturation, 20 nl = 20 ng each lipophilic dyes, benzene; circular chromatography: Petri dish, centric application, 1.5 µl = 1.5 µg each of lipophilic dyes, benzene, evaluation slit width = 2 mm.

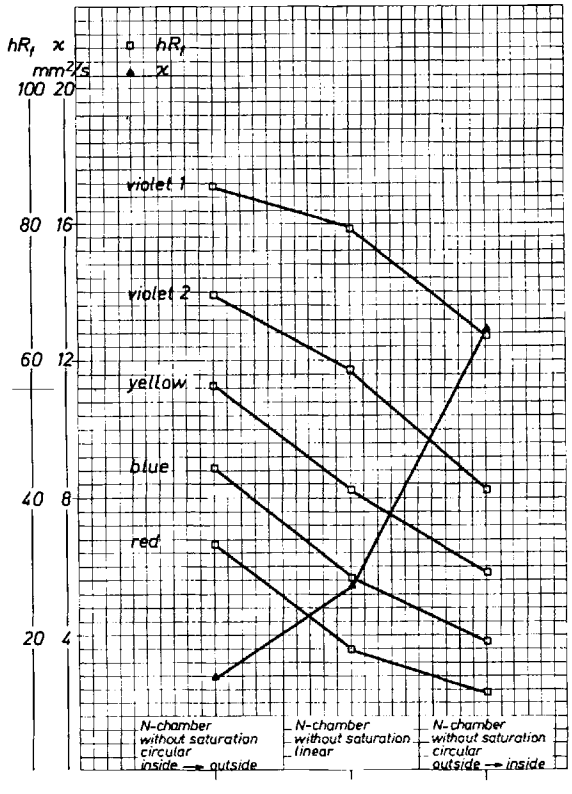

Fig. 21

- Comparison of chromatographic characteristics (hR_f values, κ values) between linear chromatography and circular chromatography, flow direction being from centre to periphery or from periphery to centre

  z_f = 80 mm; toluene.

efficiency obtained for the two methods. At a 50 mm migration distance the resolution ratio is 5.1 : 3.4 in favour of linear chromatography, which means that separation is improved by 50 %. Things are somewhat different when comparing the separation efficiency of substance-pairs with lower R_f values, i. e., the green and the blue dyes. Here a large increase in resolution with increasing migration distance is found in both chromatographic systems. With a migration distance of 20 mm, the resolution is 24 % more favourable in circular chromatography than in linear chromatography, but the figure is reduced to 3 % with a migration distance of 50 mm. These results confirm the well-known fact that circular chromatography favours the separation of substances with lower R_f values. The very much lower κ value for circular chromatography as compared with linear chromatography is notable. It amounts to no more than about one-third in the case of partially pre-loaded layers, which means that in circular chromatography development times must be about three times longer than in linear chromatography when the migration distance is the same.

In Fig. 21 a comparison is made of some chromatographic data for non-presaturated chamber systems for linear and circular chromatography, with the latter not only providing for a solvent flow from the centre to the periphery, as usual, but also from the periphery to the centre. Solvent transfer from the centre is effected via a felt wick which extends into a closed cylindrical vessel containing solvent and contacts the horizontal layer of the pre-coated HPTLC plate or of the HPTLC aluminium foil from below. Solvent transfer from the periphery takes place directly onto the aluminium foil cut in to circular shape and suitable size, cut straight from the periphery to the centre and folded so as to form a shallow cone, from which the overlapping portion is cut out as a sector secured by means of a paper clip (Fig. 22). The basic surface of this cone is matched to the ground surface of a shallow circular development chamber, which prevents solvent preloading of the coating facing upwards. Sample application is effected by means of a stationary capillary, the foil placed on a disc being rotated so that a circular or spots-on-a-circle application pattern is obtained. Both chromatographic settings constitute closed systems.

Whereas with linear chromatography the sample spot always traverses approximately identical amounts of adsorbent, in the case of circular chromatography the amount of adsorbent available for the spot changes at square level, the change being an increase in the case of chromatography from the centre to the periphery, and a decline for chromatography in the opposite direction Fig. 21 shows what had been expected, i.e., that the decline in R_f-values and the increase in κ-values practically follow a linear pattern with the three chromatographic methods: circular chromatography from centre to periphery, linear chromatography, circular chromatography from periphery to centre. The shortening of the migration time with linear chromatography as compared to circular chromatography from centre to periphery, and with circular chromatography from periphery to centre as compared to linear chromatography is an important criterion in this setting because it is not otherwise variable. The concentrating of the sample spot can be considered another advantage of circular chromatography from periphery to centre as compared to linear chromatography and, even more so, to circular chromatography from centre to periphery, where the opposite, i. e., dilution, takes place. Systematic studies with circular chromatography from periphery to centre, first described by *van Dyjk* [2] as "centripetal thin-layer chromatography", will doubtless lead to further improvements in chromatographic methods using layers.

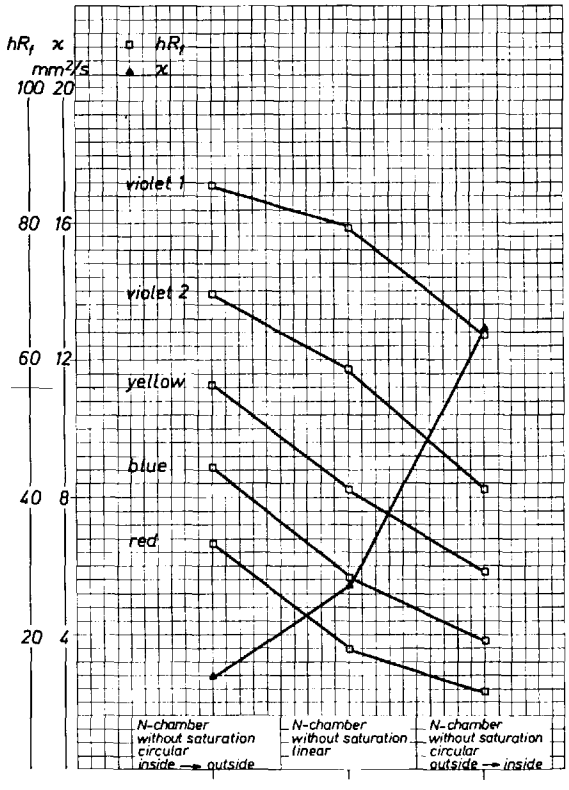

Fig. 21

- Comparison of chromatographic characteristics (hR_f values, κ values) between linear chromatography and circular chromatography, flow direction being from centre to periphery or from periphery to centre

z_f = 80 mm; toluene.

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