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THIN-LAYER CHROMATOGRAPHIC AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC READY-FOR-USE PREPARATIONS WITH CONCENTRATING ZONES*

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SUMMARY

Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) ready-for-use preparations with concentrating zones consist of a narrow inert layer of a synthetic porous silica of medium pore volume, extremely large pore diameter (*ca.* 5000 nm) and extremely small internal surface area (less than 1 m^2/g), with a very sharp interface leading to the chromatographic layer. The concentrating effect is manifested both after the application of spots (without difficulty at any level) and after large-area application by immersion, whereby it is possible to dose into the dry or wet layer. The extremely narrow line of substance that is formed at the interface provides an optimal starting situation for chromatography.

Quantitative determinations are improved, with simultaneous enhancement of the sensitivity of detection. In comparison with layers that are uniform throughout, significantly improved separation performances are attained, particularly when larger amounts are applied, and very dilute solutions and substances with lower R_F values can be used. The selection of a suitable solvent can be used to prevent or select the adsorption of salts or of polar neutral substances in the concentrating zone as desired. With direct application of urine, serum, etc., the ingredients are initially extracted in a continuous process, the so-called "clean-up", then they are concentrated, followed by chromatography. The technique is of general importance for liquid chromatography.

INTRODUCTION

The advantages of a twin-layer plate consisting of a narrow kieselguhr layer and an adjacent layer of an active sorbent were first described by Abbott and Thomson[1] in 1965, and elaborated in greater detail by Musgrave[2] in 1969. The layers are prepared by means of a spreader divided into at least two compartments. The sample is applied to the weakly adsorbing kieselguhr layer, moves with the solvent front to the silica gel layer and forms a narrow line from which chromatography can begin. According to Musgrave, the technique is based on the difference in the ad-

* This paper belongs to the section *High-performance thin-layer chromatography*.

sorbing powers of the two sorbents. Abbott and Thomson had already pointed out that the strip with the lower adsorbing power can be utilized for "clean-up", the constituents to be separated migrating with the solvent front to the more strongly adsorptive layer. In Musgrave's view this technique alleviates the problem of application of the sample solution as a narrow straight line, which can be difficult.

When a similar concentrating technique is to be applied to a homogeneous layer, then one must pre-develop to an intermediate front before chromatography by means of several runs with a strongly polar solvent, followed each time by drying. In contrast, the twin-layer technique according to Musgrave has the advantages that the chromatographic process proceeds continuously and the layer does not come into contact with a polar solvent that changes the activity of the sorbent.

DESCRIPTION OF THE PRE-COATED LAYER

The publications of Abbott and Thomson and of Musgrave have not hitherto found wide application, since the layers thus prepared, whether self-prepared or purchased ready-for-use, manifested defects that arose from the properties of the kieselguhr used and the nature of the interface between the two sorbents.

After development work relating on the one hand to the required properties and the preparation of the inert coating material and on the other hand to the coating technology required in order to attain a sharp transition zone between the two sorbents, we have been able to produce TLC and HPTLC ready-for-use preparations of silica gel 60 with concentrating zones on glass plates and on aluminium foils. The plates consist of two different layers that have a sharply defined boundary but nevertheless merge into one another so that the solvent does not meet with any resistance on its passage of the interface (Fig. 1). The layer crucial for the concentrating of substances to be separated extends across the whole width of the plate for about 25 mm in the direction of development. The layer thickness is about 150 μm. This concentrating zone consists of synthetic porous silica of medium pore volume with an extremely large pore diameter (*ca.* 5000 nm) and an extremely small internal surface area (less than 1 m^2/g). The manufacture of this layer material results in a very high degree of purity, being virtually free from heavy metals and salts. The particles of the material are narrowly classified; the mean particle size is selected such that good flow-rates are achieved. The technical and analytical expenditure on testing of this material —by physico-chemical and chromatographic methods— has been considerable. Owing to the use of the same binder as in the adjacent chromatographic layer of silica gel 60, the concentrating zone manifests the good adherence required for the application of samples. The chromatographic layer corresponds in composition and quality to the conventional layer.

CONCENTRATING EFFECT IN VARIOUS METHODS OF APPLICATION

Application of spots

If a mixture is applied with a syringe or capillary as a spot at any level on the concentrating zone, this spot spreads homogeneously in the inert layer into a circular area. If the layer is dried immediately after application, the substance is evenly distributed over the entire area, including the periphery. If, on the other hand, the

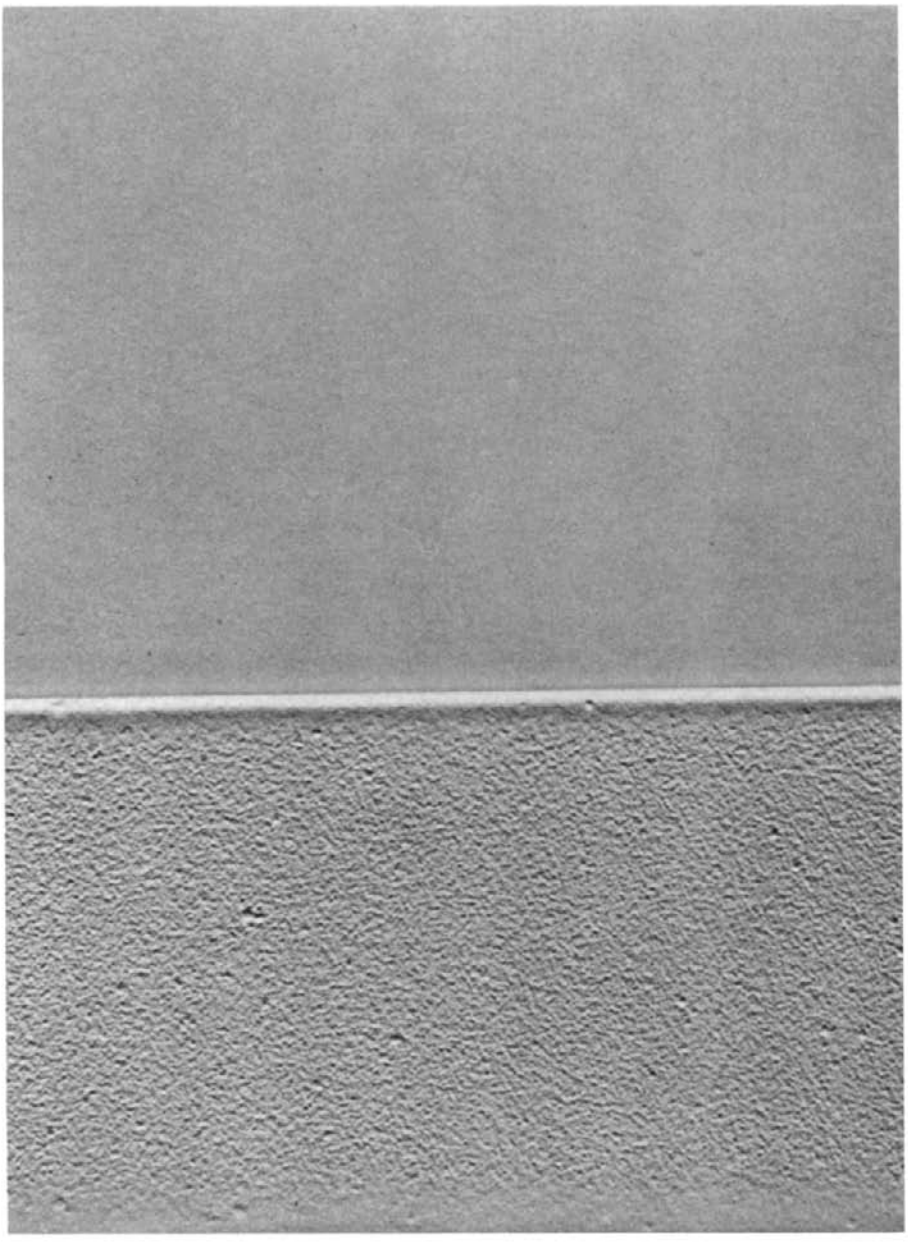

Fig. 1. Section of silica gel 60 pre-coated TLC plate with concentration zone. The white strip immediately below the sharp interface is an indentation.

layer is left to dry in the air, the excess of solvent causes chromatography to start, which leads to a slight concentrating of the substance at the margin. The substance is then concentrated at the interface into a narrow line with the length of the diameter of the circular area, and immediately chromatographed from this optimal starting

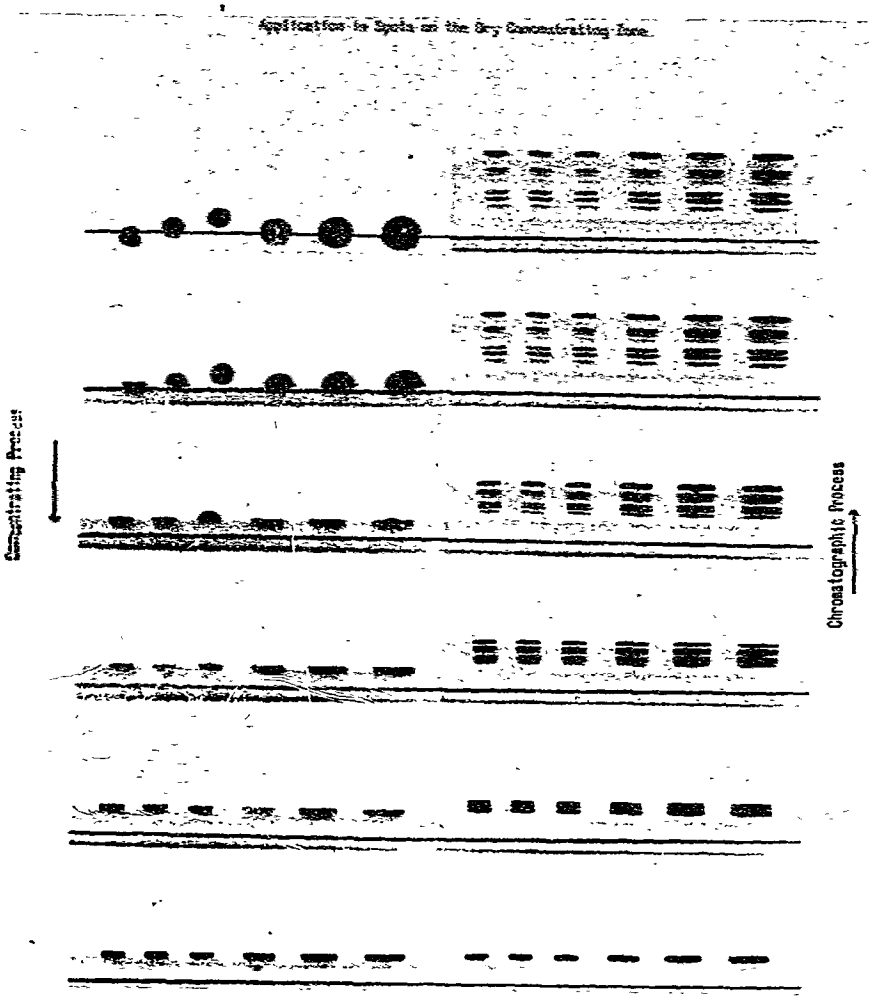

Fig. 2. Concentrating phases and chromatography phases after spot application to a dry silica gel 60 pre-coated TLC plate with a concentrating zone; mixture of seven lipophilic dyestuffs 0.1 %, volumes applied (from left to right): 4, 4, 4, 8, 12, and 16 μl; S-chamber 0.2 mm, toluene, z_f = 60 mm. The continuous black lines resulted from photography, and represent the lower boundary of the cover plate.

position. The individual phases of the concentrating and of the chromatography processes are clearly recognizable in Fig. 2. The phase of highest concentrating is at the start of the chromatography. The optimal dosage quality Q_D specified by Kaiser[3,4] is automatically achieved here.

Identical R_F values result over the entire layer, independent of the level of the concentrating zone at which the samples are applied. The constancy of the R_F values is determined solely by the precision of the interface. In serial experiments, the saving in time due to the less care that is necessary in application of the samples is considerable.

Volumes between 2 and 20 μl have been applied, equivalent to 2 and 20 μg of each substance (Fig. 3). Increasing the volume applied results in larger diameters of

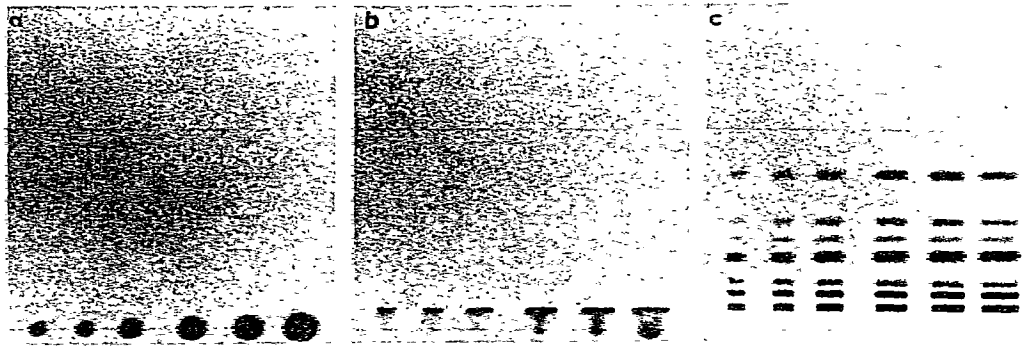

Fig. 3. (a) Application phase, (b) concentrating phase, and c) chromatography phase; mixture of seven lipophilic dyestuffs 0.1%; volumes applied (from left to right): 2, 4, 8, 12, 16, and 20 μl; N-chamber without chamber saturation, toluene, z_f = 100 mm.

TABLE I

CONCENTRATING EFFECT WITH VARIOUS AMOUNTS APPLIED ON SILICA GEL 60 PRE-COATED TLC PLATES AND ON SILICA GEL 60 PRE-COATED HPTLC PLATES WITHOUT AND WITH CONCENTRATING ZONES

All of the measurements were carried out with a Zeiss PMQ II chromatogram spectrophotometer in the reflection mode, the analogue output being connected to an IBM System 7 process computer.

	Amount applied	Silica gel Zone after Application			Concentrating Zone after Application			Interface after Concentrating Proc.			
		Diameter of the Circular Area	Circular Area		Diameter of the Circular Area	Circular Area		Width	Length	Area	Area Rel.
	μl	mm	absolute mm^2	Relation	mm	absolute mm^2	Relation	mm	mm	mm^2	
TLC-Silica gel/ TLC-Silica gel with Conc.Zone	4	6,0	28,3	100	10,5	86,6	306	0,8	10,5	8,4	29,7
	8	8,0	50,3	100	14,0	153,9	306	1,0	14,0	14,0	27,8
	12	9,5	70,9	100	16,5	213,8	302	1,0	16,5	16,5	23,3
	16	10,5	86,6	100	17,5	240,5	278	1,0	17,5	17,5	20,2
	20	11,5	103,9	100	20,0	314,2	302	1,2	20,0	24,0	23,1
Mean Value of the Relations				100 4,0			299 12,1				24,8 1
HPTLC-Silica gel/ HPTLC-Silica gel with Conc.Zone	2	4,0	12,6	100	9,0	63,6	505	0,6	9,0	5,4	42,9
	4	5,5	23,8	100	12,0	113,1	475	0,8	12,0	9,6	40,3
	8	7,5	44,2	100	15,0	176,7	400	1,0	15,0	15,0	33,9
Mean Value of the Relations				100 2,6			460 11,8				39,0 1

the circular areas and greater lengths of the lines after concentrating. The tailing of the spots that is visible on application of the larger volumes and single development up to the interface is eliminated through the greater flow of solvent during chromatography, and has no disadvantageous influence on the separation performance.

The concentrating effect for various volumes applied to TLC plates and HPTLC plates is represented in Table I. In comparison with the application to the concentrating zone there is a 12-fold concentrating at the interface. Compared with the application on to the silica gel zone, the concentration with respect to area is thus still four-fold with the TLC plate and three-fold with the HPTLC plate. The width of the concentrated lines, and not the total area, however, is critical for the chromatographic performance. Even when large amounts are applied, the width is usually less than 1 mm.

If the application as spots by means of syringe or capillary is not in portions but with drying after each application, then the concentration distributions within the substance lines vary. As may be seen from Fig. 4 and from measurements perpendicular to the direction of the run, the maximum concentrations of the substance on application without intermediate drying are to be found in the middle of the peak area; however, on application with intermediate drying they are located at the margins of the peak area (Figs. 5 and 6). With ideally even applications the peak areas are symmetrical in each instance, but this is not always attained in practice by the method of intermediate drying. With the measured values in Table II, also the diameters of the circular areas formed in each instance with and without intermediate drying are given for the applied amounts of 10 and 20 μl, a ring of stronger concentration having

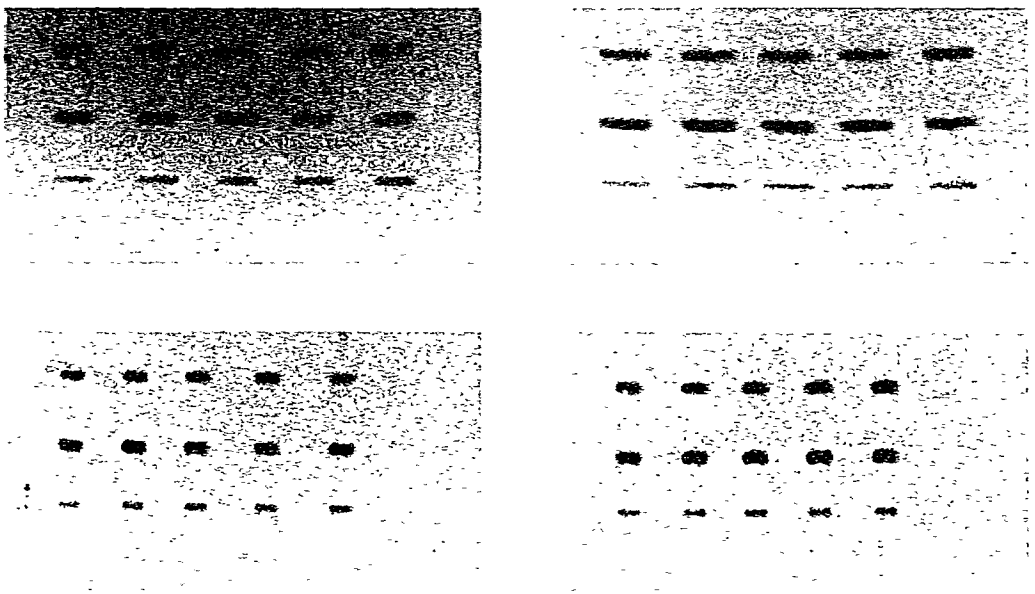

Fig. 4. Chromatograms of a mixture of three lipophilic dyestuffs 0.1 %; silica gel 60 pre-coated TLC plates with concentrating zone; amount of solution applied in each instance 10 μl (left) or 20 μl (right), without intermediate drying (above) and with intermediate drying (below); normal chamber without chamber saturation, toluene, z_f = 100 mm.

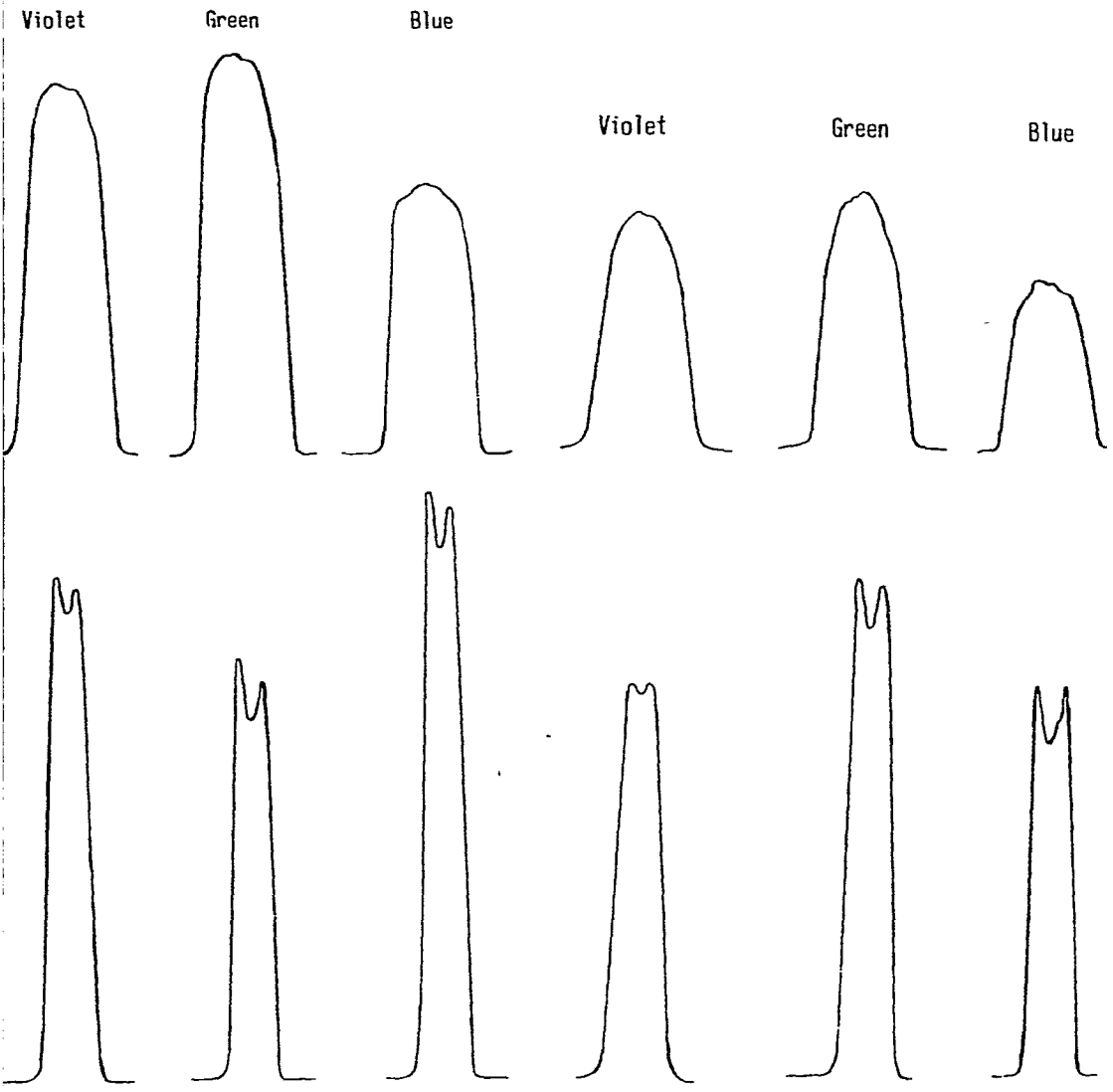

Fig. 5 Fig. 6

Fig. 5. Peak-area profiles of three lipophilic dyestuffs after chromatography on silica gel 60 precoated TLC plates with a concentrating zone, amount of 0.1 % solution applied in each instance 20 μl, without intermediate drying (above) and with intermediate drying (below); N-chamber without chamber saturation, toluene, z_f = 100 mm, measurements perpendicular to direction of development.

Fig. 6. Peak-area profiles of three lipophilic dyestuffs after chromatography on silica gel 60 precoated TLC plates with a concentrating zone, amount of 0.01 % solution applied in each instance 10 μl, without intermediate drying (above) and with intermediate drying (below); N-chamber without chamber saturation, toluene, z_f = 100 mm, measurements perpendicular to direction of development.

TABLE II

PEAK AREAS AND PEAK-AREA RATIOS WITH VARIOUS VOLUMES APPLIED AND VARIOUS MODES OF APPLICATION ON SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.1%; N-chamber without chamber saturation, toluene, z_f = 100 mm, measurements perpendicular to direction of development.

	Diameter of the Circular Area after Application [mm]	Width of the Bands [mm]			Peak Areas [mVs]				Relations of the Peak Areas [%]			
		V	G	B	V 550nm	G 630nm	B 586nm	Mean Value	V/G	G/B	B/V	Mean Value
Application without Drying												
10 µl = 10 µg		21,2	20,7	18,9	9640	12480	8148		77,24	153,17	84,52	
		21,3	20,9	19,0	9355	12319	7870		75,94	156,53	84,13	
		20,9	20,4	18,7	8645	11734	7618		73,67	154,03	88,12	
		21,3	20,7	19,0	8885	11613	7784		76,51	149,19	87,61	
		22,5	21,4	19,2	8942	12097	8067		73,92	149,96	90,21	
$\bar{x}$	d_1= 15,5											
	d_2= 15,3	21,4	20,8	18,9	9093	12049	7897		75,46	152,58	86,92	
s					398	371	214		1,59	3,02	2,55	
s%					4,38	3,08	2,71	3,39	2,10	1,98	2,95	2,34
20 µl = 20 µg		26,5	26,1	24,0	22782	23228	16371		98,08	141,89	71,86	
		27,0	26,3	24,7	23877	25417	16832		93,94	151,00	70,49	
		26,7	26,5	24,2	22958	25161	16598		91,24	151,59	72,30	
$\bar{x}$	d_1= 20,1											
	d_2= 19,9	26,7	26,3	24,3	23206	24602	16600		94,42	148,16	71,55	
s					588	1197	231		3,45	5,44	0,94	
s%					2,53	4,86	1,39	2,93	3,65	3,67	1,32	2,88
Application with Drying												
10 µl = 10 µg		15,0	14,5	13,0	12343	13204	8854		93,48	149,13	71,73	
		15,0	14,5	13,1	12286	13362	8772		91,95	152,33	71,40	
		15,1	14,6	12,6	11933	13075	8698		91,27	150,32	72,89	
		14,7	14,1	12,8	11439	12674	8659		90,26	146,37	75,70	
$\bar{x}$	d_1= 6,2											
	d_2= 5,2	15,0	14,4	12,9	12000	13079	8746		91,74	149,54	72,93	
s					416	294	86		1,35	2,49	1,95	
s%					3,46	2,25	0,98	2,23	1,47	1,67	2,68	1,94
20 µl = 20 µg		15,5	14,5	13,4	15252	17228	11598		88,53	148,54	76,04	
		15,3	14,5	13,3	15950	17828	11845		89,47	150,51	74,26	
		15,5	15,0	13,7	16533	18401	12301		89,85	149,59	74,40	
		15,5	15,3	13,7	16402	18388	12217		89,20	150,51	74,48	
		15,6	14,5	13,2	15596	16799	11707		92,84	143,50	75,06	
$\bar{x}$	d_1= 7,3											
	d_2= 6,3	15,4	14,7	13,4	15947	17729	11934		89,98	148,53	74,85	
s					538	709	311		1,67	2,93	0,73	
s%					3,37	4,00	2,61	3,33	1,86	1,97	0,98	1,60

formed between the diameters d_1 and d_2. The concentration distributions of the lines formed at the interface from the circular areas were calculated by integration of the segments of the circular areas, a five-fold increase in the outer ring concentration having been assumed. The profiles thus calculated are represented in Fig. 7, and they agree in principle with the measured results in Fig. 5.

Fig. 8 records other profiles calculated for peak areas; the horizontal row 1 shows the initial circular areas with concentration rings of varying widths, and rows 2, 3 and 4 show varying concentration ratios. All of the profiles are symmetrical. The maximum concentration distribution in the middle of the peak is attained only with

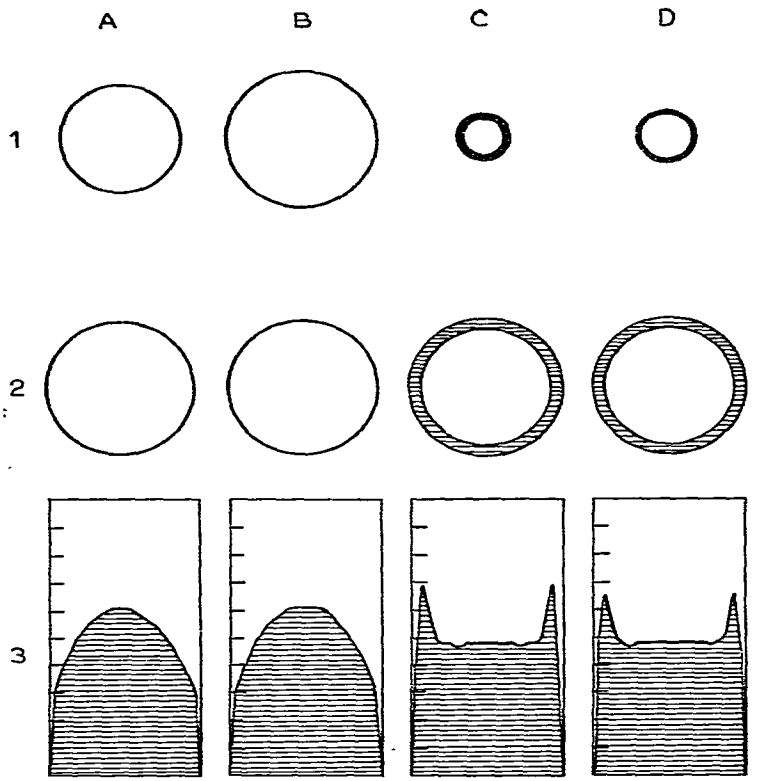

Fig. 7. Peak area profiles at the interface, calculated from the diameters of the circular areas after application as listed in Table II; A, 10 μl, B, 20 μl applied, in each instance without intermediate drying, C, 10 μl, D, 20 μl applied, in each instance with intermediate drying; 1, circular areas on application in relation to the diameter measured, 2, circular areas after application, referred to the same diameter, 3, peak area profiles at the interface, calculated from the former, assuming a five-fold increase in ring concentration.

a very narrow, and hence weak, concentration ring (A2, B2). The other peak profiles exhibit a dumb-bell-shaped concentration distribution. For quantitative measurements, it is important to have profiles that are constant over a wide region in the middle of the peak, such as C2, D2, B3, C3 and B4. It is noteworthy that despite the differences in development of profiles induced by the variety of methods of application, the concentrations found agree over a wide region in the centre of the profile. Assuming that the peak-area profiles are evaluated exactly in the middle between 25% and 75% of the total width, the following values are obtained on the basis of a total concentration of 100:

for the profiles B2 to F2: $\bar{x} = 53.2$, $s = 1.35$, $s(\%) = 2.5$
B3 to F3: $\bar{x} = 48.7$, $s = 1.75$, $s(\%) = 3.6$
B4 to F4: $\bar{x} = 45.5$, $s = 2.54$, $s(\%) = 5.6$

On using layers with concentrating zones the concentration distribution of the lines is theoretically not so ideal as with an exact application in the form of narrow lines. This does not, however, reduce the efficiency of the method.

Large-area application by immersion

If it is desired to detect very small amounts of substances in very dilute solutions, or to isolate small amounts of substances with the aim of identifying them, then the plates with the concentrating zone are dipped into the solution of the mixture. The solution can be contained in a shallow trough or another suitable vessel. The maximum depth of immersion must not, of course, exceed the height of the concentrating zone. In order to apply larger amounts the dipping process can be repeated one or more times after brief drying in each instance.

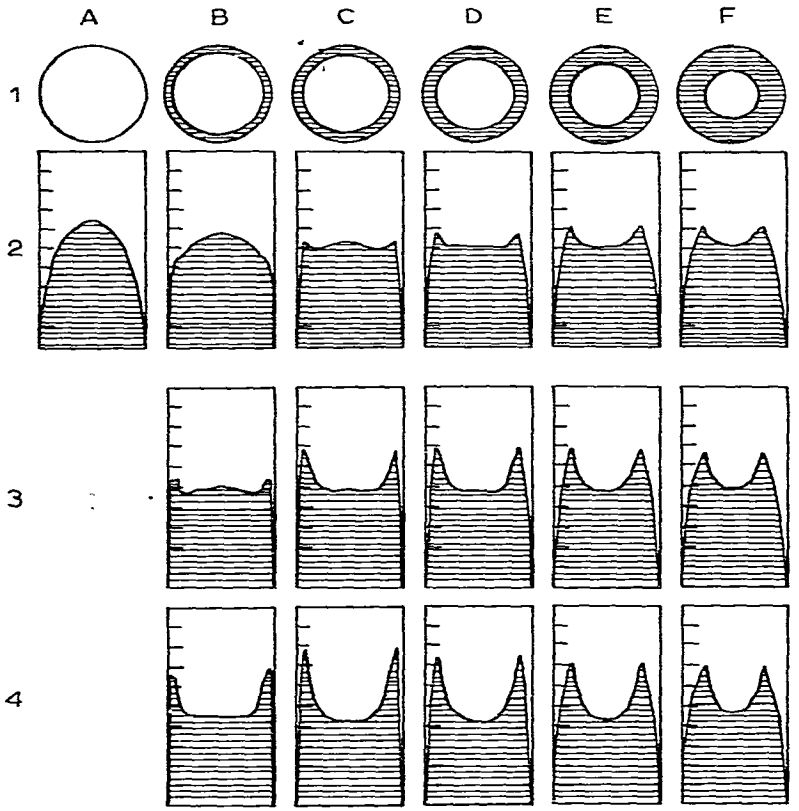

Fig. 8. Calculated peak area profiles; 1, circular areas on application. A, without ring of increased intensity; B–F, diameter of the respective rings with 10–50% increased intensity of the overall diameter. 2–4, peak area profiles at the interface with 2.5-fold, 5-fold and 10-fold intensity ratio, respectively.

The concentrating and the immediate subsequent chromatography are illustrated in Fig. 9. Owing to the precision of the interface, the state of narrowest line width is passed through for a few seconds. This effect is responsible for the high chromatographic performance. With large-area application by immersion, the substance concentration is evenly distributed over the entire narrow line. A better application in the form of narrow lines cannot be attained with an automatic applicator. When very dilute solutions are used, it is necessary to make several applications in the form of

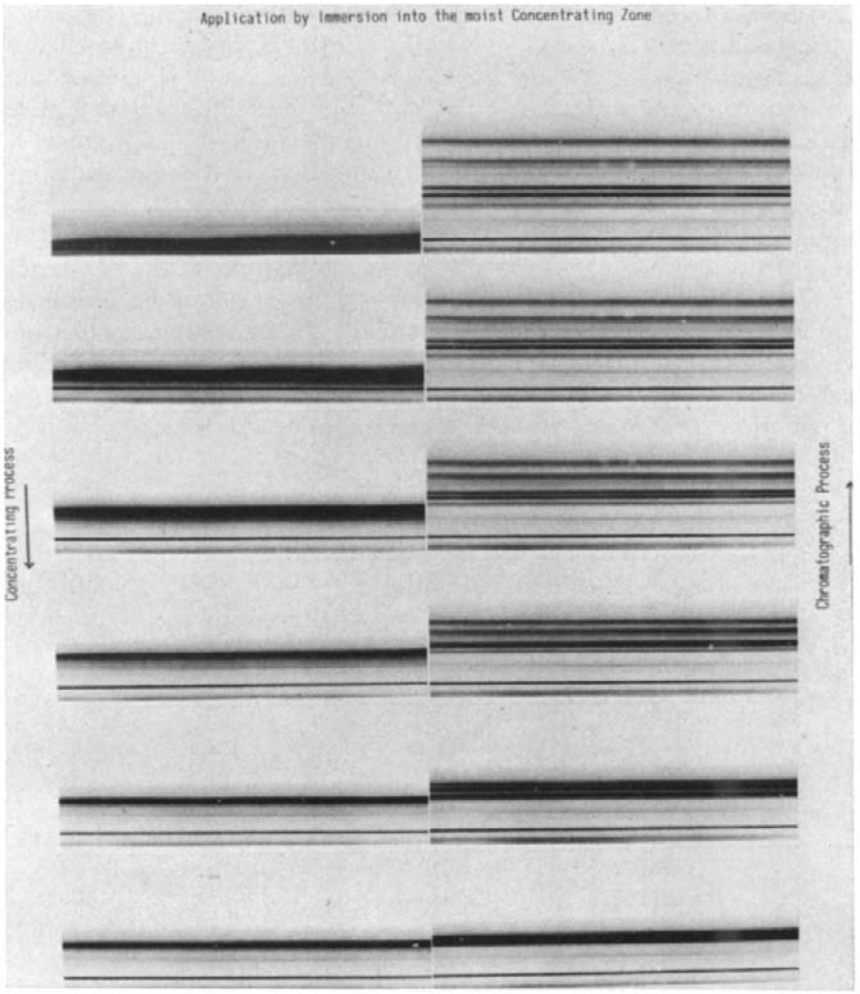

Fig. 9. Concentrating phases and chromatography phases after application by immersion into the solvent-moistened layer of silica gel 60 pre-coated TLC plate with a concentrating zone; mixture of seven lipophilic dyestuffs 0.1%, N-chamber, 0.2 mm, toluene, $z_f = 60$ mm.

narrow lines, with intermediate drying in each instance, in order to bring about the chromatographic development of the same amount of substance.

If the method of application by immersion with conventional silica gel plates without a concentrating zone is used, then concentrating into a band or a narrow line is attainable only if the plate is developed several times with a strongly polar solvent up to a new front about 10 or 20 mm above the level of immersion, before proceeding with chromatography. As can be seen from the data in Table I, the normal silica gel TLC layer on immersion takes up a volume of substance solution about three times larger than that with the concentrating zone.

Wet application

Kaiser[5,6] was the first to set forth the advantages of wet dosage, in conjunction

with the U-chamber. As on employment of layers with concentrating zones the exact position of application is not critical, such layers also afford the opportunity of wet dosage without risk of subsequent uneven R_F values. Development is carried out to the level of the interface in the solvent selected, followed by removal of the plate from the chamber, and dosage either by the application of spots with a syringe or capillary, or by immersion into the wet layer. As with dry dosage, the concentrating effect starts after the plate has been placed in the eluent and is followed by chromatography.

An advantage of wet dosage is that the risk of decomposition of the substance on application is still further reduced. In particular, however, the flow behaviour is favoured by the development of a very even solvent front. In Fig. 9 the application was effected by immersion in the briefly pre-developed wet concentrating zone. The evenly formed lines of substances extending up to the lateral margins of the layer result from this application technique. After large-area application by immersion, it

TABLE III

PEAK AREAS AND PEAK AREA RATIOS WITH VARIOUS VOLUMES APPLIED ON SILICA GEL 60 PRE-COATED TLC PLATES AND SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.1%; N-chamber without chamber saturation, toluene, z_f = 100 mm, measurements perpendicular to direction of development.

		Width of the Bands [mm]			Peak Areas [mVs]				Relations of the Peak Areas [%]			
		V	G	B	V 550nm	G 630nm	B 586nm	Mean Value	V/G	G/B	B/V	Mean Value
TLC-Silica gel	$\bar{x}$	7,6	7,1	5,4	415	598	463		69,32	129,34	111,68	
200nl = 200 ng	s				4	10	28		0,55	5,76	5,88	
	s%				0,85	1,66	6,11	2,87	0,80	4,46	5,26	3,51
	$\bar{x}$	8,2	6,8	5,4	447	669	490		66,84	136,52	109,68	
	s				8	11	16		0,61	4,78	3,05	
	s%				1,78	1,60	3,24	2,21	0,91	3,50	2,78	2,40
Mean Value	s%				1,32	1,63	4,68	2,54	0,86	3,98	4,02	2,95
TLC-Silica gel	$\bar{x}$	17,6	16,8	15,3	7581	8974	7120		84,71	126,09	93,96	
with Conc.Zone	s				203	528	79		6,00	8,41	3,10	
5 µl = 5 µg	s%				2,68	5,88	1,10	3,22	7,08	6,67	3,30	5,68
	$\bar{x}$	17,2	16,5	14,9	7392	9392	6698		78,71	140,25	90,63	
	s				78	167	93		0,79	4,31	1,96	
	s%				1,05	1,78	1,39	1,41	4,00	3,07	2,16	3,08
Mean Value	s%				1,87	3,83	1,25	2,32	5,54	4,87	2,73	4,38
TLC-Silica gel	$\bar{x}$	20,4	20,1	18,9	13365	14950	9455		89,40	158,13	70,76	
with Conc.Zone	s				162	199	174		0,78	1,77	1,39	
10µl = 10µg	s%				1,22	1,33	1,84	1,46	0,87	1,12	1,96	1,32
	$\bar{x}$	20,5	20,1	18,7	12920	14753	9372		87,59	157,43	72,57	
	s				316	362	181		1,49	2,80	2,19	
	s%				2,45	2,46	1,94	2,28	1,70	1,78	3,02	2,17
Mean Value	s%				1,84	1,90	1,89	1,87	1,29	1,45	2,49	1,74

is still possible to apply reference substances in the form of spots. The subsequent assignment of the lines is thus facilitated.

QUANTITATIVE EVALUATIONS

Tables III and IV give the mean peak areas with standard deviations in the case of measurements perpendicular to the direction of development between pre-coated TLC and HPTLC plates without and with a concentrating zone. Of the blue, green and violet dyestuffs with low, medium and high R_F values, the line widths increase slightly on application to the concentrating zone because of diffuse spreading. With the same application technique the line widths on application of 10 μl to the TLC pre-coated plate with a concentrating zone are, of course, larger than on application of 5 μl (Table III). The smaller line width with the application of 2 μl to the HPTLC plate with a syringe in comparison with a capillary with concentrating zone is explained by the slower draining when using the capillary, the solvent evaporating during the application (Table IV).

TABLE IV

PEAK AREAS AND PEAK AREA RATIOS WITH VARIOUS VOLUMES APPLIED ON SILICA GEL 60 PRE-COATED HPTLC PLATES AND SILICA GEL 60 PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.1%; N-chamber without chamber saturation, toluene, z_f = 50 mm, measurements perpendicular to direction of development.

		Width of the Bands [mm]			Peak Areas [mVs]				Relations of the Peak Areas [%]			
		V	G	B	V 550nm	G 630nm	B 586nm	Mean Value	V/G	G/B	B/V	Mean Value
HPTLC-Silica gel	$\bar{x}$	5,3	3,8	3,0	321	371	302		86,60	122,83	94,09	
50 nl = 50 ng	s				5	8	10		2,05	2,06	3,30	
	s%				1,54	2,15	3,47	2,39	2,37	1,68	3,51	2,52
HPTLC-Silica gel	$\bar{x}$	12,3	12,0	11,2	5849	7198	5449		81,27	132,11	93,16	
with Conc.Zone	s				135	238	119		2,01	3,05	0,81	
2 μl = 2 μg	s%				2,30	3,30	2,18	2,59	2,47	2,31	0,87	1,88
Capillary	$\bar{x}$	12,4	12,3	11,2	5269	6876	4136		75,65	166,27	78,52	
	s				196	307	157		1,45	2,37	1,30	
	s%				3,73	4,46	3,81	4,00	1,89	1,43	1,66	1,66
Mean Value	s%				3,02	3,88	3,00	3,30	2,18	1,87	1,27	1,77
HPTLC-Silica gel	$\bar{x}$	11,7	11,4	10,7	5194	6148	3832		84,48	160,39	73,85	
with Conc.Zone	s				245	270	94		1,04	4,06	2,06	
2 μl = 2 μg	s%				4,71	4,39	2,44	3,85	1,23	2,53	2,79	2,18
Syringe	$\bar{x}$	11,7	11,3	10,4	5235	6006	4018		87,18	149,57	76,73	
	s				202	260	179		0,61	2,15	1,38	
	s%				3,85	4,33	4,46	4,21	0,70	1,44	1,80	1,31
Mean Value	s%				4,28	4,36	3,45	4,03	0,97	1,99	2,30	1,75

Comparison of the pre-coated TLC plate with 200 nl applied and the pre-coated TLC plate with a concentrating zone and volumes of 5 and 10 μl applied (Table III) reveals that the standard deviations of the peak areas are slightly improved from 2.5 to 2.3 and 1.9%. However, the standard deviations of the peak-area ratios (internal standard) at 3.0, 4.4 and 1.7% are only improved with the 10-μl application. Comparison of the pre-coated HPTLC plate with 50 nl applied and the HPTLC plate with a concentrating zone and 2 μl applied with a capillary or with a syringe (Table IV) shows that the standard deviations of the peak areas deteriorated from 2.4 to 3.3 and 4.0%. However, the standard deviations of the peak-area ratios improved from 2.5 to 1.8% in each instance. However, as the application of larger amounts (microlitre volumes) is generally less difficult than the application of smaller amounts (nanolitre volumes), plates with concentrating zones offer an advantage which should not be under-estimated.

In Tables II and V the peak-area measurements made perpendicular to the direction of development on pre-coated TLC plates with a concentrating zone in the case of application without and with intermediate drying when using a 0.1% and a 0.01% solution are compared. The corresponding area profiles measured are repre-

TABLE V

PEAK AREAS AND PEAK AREA RATIOS WITH VARIOUS VOLUMES APPLIED ON SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.01%; N-chamber without chamber saturation, toluene, z_f = 100 mm, measurements perpendicular to direction of development.

		Diameter of the Circular Area after Application [mm]	Width of the Bands [mm]			Peak Areas [nVs]				Relations of the Peak Areas [%]			
			V	G	B	V 550nm	G 630nm	B 586nm	Mean Value	V/G	G/B	B/V	Mean Value
TLC-Silica gel with Conc.Zone 10µl = 1 µg Application without Drying													
			20,0	17,6	16,7	3126	3210	2100		97,38	152,86	67,18	
			19,7	18,2	17,4	3172	3320	2341		95,54	141,82	73,80	
			20,0	17,7	16,9	3069	3079	2086		99,68	147,60	67,97	
			20,0	17,4	16,5	3132	3362	2360		93,16	142,46	75,35	
	$\bar{x}$	16,8	19,9	17,7	16,9	3125	3203	2222		96,44	146,19	71,08	
	s					42	121	149		2,77	5,15	4,10	
	s%					1,36	3,77	6,71	3,95	2,87	3,52	5,77	4,05
Application with Drying			12,9	11,5	10,2	3119	3592	2721		86,83	132,01	87,24	
			12,5	10,9	10,0	3104	3505	2689		88,56	130,35	86,63	
			12,8	11,3	9,9	3143	3544	2726		88,69	130,01	86,73	
			12,8	11,3	10,1	3155	3614	2753		87,30	131,27	87,26	
	$\bar{x}$	7,9	12,8	11,2	10,1	3130	3564	2722		87,85	130,91	86,97	
	s					23	49	26		0,92	0,91	0,33	
	s%					0,74	1,37	0,96	1,02	1,05	0,70	0,38	0,71

sented in Figs. 5 and 6. The reduction in line widths when equal amounts are applied and on dosage with intermediate drying in comparison with dosage without intermediate drying is considerable. When the 0.1% solution is employed, the standard deviations of the peak areas measured have approximately the same value for the two methods of application, but those of the peak-area ratios (internal standard) are favourable for the application with intermediate drying (Table II). With the 0.01% solution, however, all of the standard deviations are markedly improved for the method of application with intermediate drying, namely from 4.0 to 1.0% and from 4.1 to 0.7% (Table V). These results show that with quantitative evaluation a certain minimum concentration of substance per unit area is necessary. The higher substance loading attained by application with intermediate drying thus yields the more favourable standard deviation.

Table VI and Fig. 10 list the results of quantitative peak area measurements with volumes applied varying from 2 to 6 μl and substance concentrations varying from 0.01 to 0.1%, evaluated this time in the middle, in each instance in the direction of development with smaller areas in comparison with the lines. The same mean wavelength of 586 nm was used for the violet, green and blue dyestuffs. For a constant substance concentration the ratios of the peak areas attained (internal standard) with various volumes applied are in good agreement. The regression lines run linearly with increasing gradient at higher volumes applied, and exhibit correspondingly high regression coefficients.

TABLE VI

PEAK AREAS AND PEAK AREA RATIOS WITH VARIOUS VOLUMES APPLIED AND VARIOUS CONCENTRATIONS ON SILICA GEL 60 PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs, N-chamber without chamber saturation, toluene, z_f = 50 mm, measurements perpendicular to direction of development in centre of the peaks, area measured 6 × 0.2 mm.

Volume applied	Concentration of the Solution	Amount applied	Diameter of the Circular Area after Application	Peak Areas [mVs]			Relations of the Peak Areas [%]		
				V	G	B	V/G	G/B	B/V
µl	%	µg	mm	586 nm	586 nm	586 nm			
2	0,01	0,2	10	230	421	267?	54,63		
2	0,05	1	10	905	1449	1689	62,46	85,79	186,63
2	0,1	2	10	2047	3086	3217	66,33	95,93	157,16
4	0,01	0,4	13	333	588	480?	56,63		
4	0,05	2	13	1242	2040	2297	60,88	88,81	184,94
4	0,1	4	13	2706	4146	4099	65,27	101,15	151,48
6	0,01	0,6	16	358	642	378?	55,76		
6	0,05	3	16	1448	2364	2629	61,25	89,92	181,56
6	0,1	6	16	3108	4702	4463	66,10	105,36	143,60

This result was to be expected on the basis of the calculations on p. 831, which yielded a good agreement between various profile concentrations in the middle of the lines. Thus, on application of the same volumes of solutions of varying concentrations in each instance, it is possible without difficulty to measure the peak areas in the direction of development in the middle of the lines; the length of the slit of the photometer should be about half the length of the line. The use of layers with concentrating zones not only improves quantitative determinations, but also simultaneously enhances the sensitivity of detection of extremely dilute solutions.

With volumes applied in the ratio 1:10 (2 μl to 20 μl) and with 1:100 concentration of the solutions (0.001 % to 0.1 %), amounts of substance in the ratio 1:1000 (0.02 μg to 20 μg) are quantitatively detectable on the same plate without difficulty.

COMPARISON OF CHROMATOGRAPHIC PERFORMANCE

The separation number recommended by Kaiser[3,4] for assessment of separation performance has in our opinion proved to be of outstanding value as a substance-independent parameter in TLC. We are able to confirm the high degree of reproducibility in several hundred determinations.

By definition, the separation number gives the maximum number of completely separated substances in the range from $R_F = 0$ to $R_F = 1$, a pair of substances being considered to be completely separated when the interval between the concen-

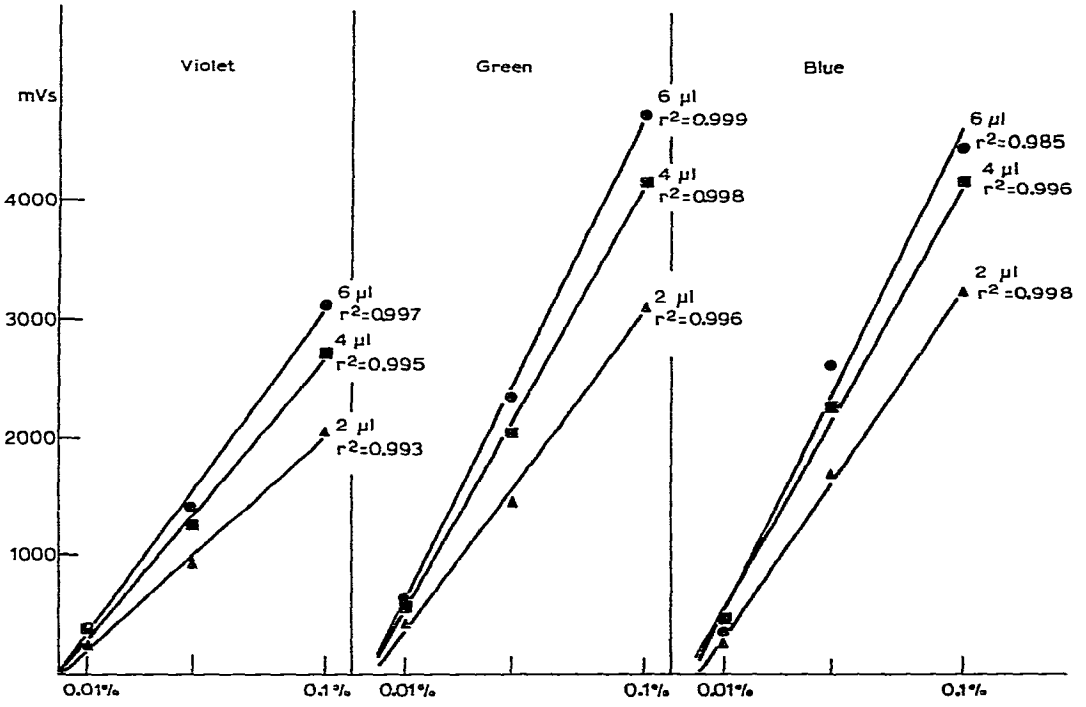

Fig. 10. Regression lines of chromatographically separated lipophilic dyestuffs at volumes applied of 2, 4, and 6 μl; for experimental conditions, see Table VI.

tration maxima corresponds to the sum of the two peak widths at half-height. The following relationship is used to calculate the separation number:

$$SN = \frac{z_f}{b_0 + b_1} - 1$$

where

z_f (mm) = distance run by solvent from start to front

b_0 (mm) = extrapolated width of starting spot at half-height of the individual concentration curve

b_1 (mm) = extrapolated width of the spot at half-height of the individual concentration curve at $R_F = 1$

Table VII and Fig. 11 compare the separation numbers with varying volumes applied for chromatography on conventional silica gel 60 pre-coated TLC plates and on silica gel 60 pre-coated TLC plates with a concentrating zone at a z_f value of 100 mm. It is apparent from the exponential curves and the relative values of the respective separation numbers that the advantage of the plate with a concentrating

TABLE VII

SEPARATION NUMBERS WITH VARIOUS AMOUNTS APPLIED FOR SILICA GEL 60 PRE-COATED TLC PLATES AND SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE

Mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_f = 100 mm.

Amount applied µg	Separation Number TLC – Silica gel	TLC – Silica gel with Conc.Zone	Relation of the Separation Numbers between TLC-Silica gel with Conc.Zone and TLC-Silica gel
0,01	11,22	12,83	1,14
0,1	11,20	12,82	1,14
1	11,02	12,75	1,16
2	10,82	12,68	1,17
3	10,63	12,61	1,19
4	10,44	12,54	1,20
5	10,25	12,46	1,22
10	9,37	12,11	1,29
20	7,82	11,43	1,46
30	6,53	10,79	1,65
40	5,45	10,19	1,87
50	4,55	9,62	2,11
100	1,85	7,22	3,90

zone lies mainly in the larger amounts that can be applied. Whereas with amounts applied of 10 or 100 ng the improvement in the separation performance is only 15%, with 10 μg the improvement is 30%, and with 50 μg more than 100%. The separation number attained even with a volume applied of 100 μl, corresponding to 100 μg of each substance, in a run of 100 mm is remarkably high, namely 7.

Table VIII and Fig. 12 compare the separation numbers attained on silica gel 60 pre-coated TLC plates with a concentrating zone for the application of the same volume in each instance of two solutions of different concentrations. In comparison with the 0.1% solution, the improvement in separation performance with the 0.01% solution is detectable in the range from 10 to 20%, and is only slightly influenced by the absolute volume applied.

It can be seen from Table IX that, despite correct measurement of the hR_F values from the respective starting zone, *i.e.*, on the one hand from the starting point and on the other from the interface, the hR_F values are slightly higher for the plate with a concentrating zone than for the conventional plate with a uniform layer throughout. The reason is not readily apparent. As the difference is more marked at lower R_F values than at higher values, it could possibly be the prior development effect within the concentrating zone that is responsible.

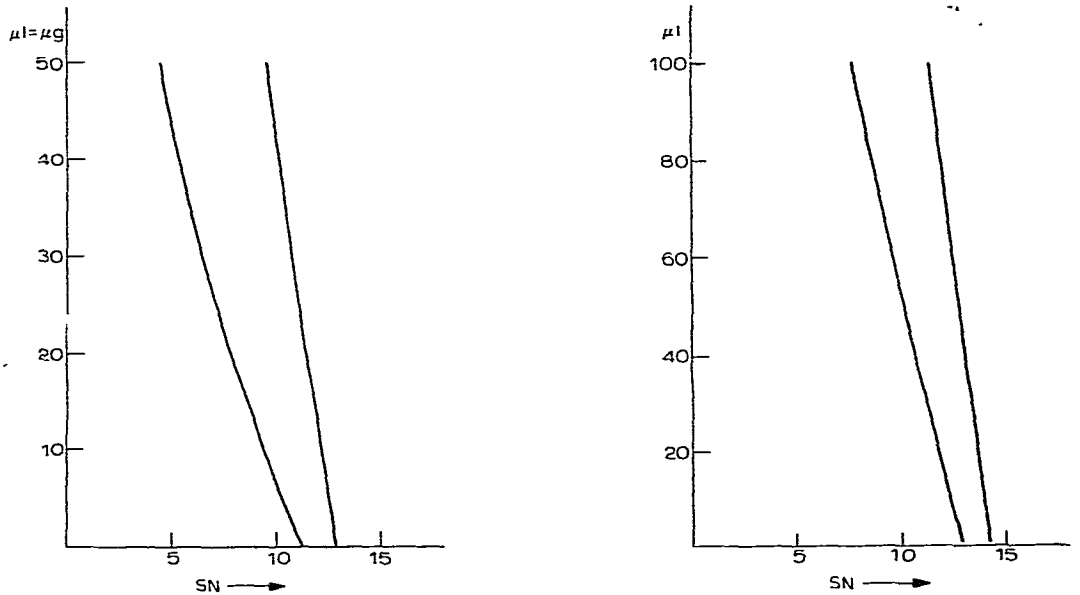

Fig. 11. Separation numbers *versus* amounts applied on silica gel 60 pre-coated TLC plates (left) and silica gel 60 pre-coated TLC plates with concentrating zone (right); for experimental conditions, see Table VII.

Fig. 12. Separation numbers *versus* amounts applied of solutions of varying concentrations; 0.1% (left), 0.01% (right); for experimental conditions, see Table VIII.

Table X and Fig. 13 show the separation numbers for various amounts applied using silica gel 60 pre-coated HPTLC plates and silica gel 60 pre-coated HPTLC plates with a concentrating zone at a z_f value of 50 mm. Here also there is the same tendency

TABLE VIII

SEPARATION NUMBERS WITH VARIOUS AMOUNTS APPLIED OF SOLUTIONS OF VARIOUS CONCENTRATIONS

Silica gel 60 pre-coated TLC plates with a concentration zone, mixture of three lipophilic dyestuffs, N-chamber without chamber saturation, toluene, $z_f = 100$ mm.

Amount applied µg	Separation Number 0,1% solution	0,01% solution	Relation of the Separation Numbers between the 0,01% and the 0,1% solutions
0,01	12,92	14,23	1,10
0,1	12,91	14,23	1,10
0,5	12,89	14,22	1,10
1	12,86	14,20	1,10
2	12,79	14,17	1,11
3	12,73	14,14	1,11
4	12,66	14,11	1,11
5	12,60	14,08	1,12
6	12,53	14,05	1,12
7	12,47	14,02	1,12
8	12,41	13,99	1,13
9	12,35	13,96	1,13
10	12,28	13,92	1,13
15	11,98	13,77	1,15
20	11,68	13,62	1,17
25	11,39	13,47	1,18
50	10,03	12,75	1,27
100	7,79	11,42	1,47

as with the TLC plates. With increasing amounts applied, the difference in separation efficiency in favour of the plate with the concentrating zone is markedly greater. Comparison of the TLC grades with the HPTLC grades reveals that the increase in separation performance with the HPTLC plate with the concentrating zone is greater. With very small amounts applied (about 1 µl) it is 40%, and at 10 µl 80%. Even with a volume applied of 25 µl, corresponding to 25 µg of each substance, an HPTLC layer with a concentrating zone can still separate eight substances in a run of 50 mm.

It can be seen from Table XI that here also the hR_F values for the layer with the concentrating zone are slightly higher than those for the pure silica gel layer, especially at lower hR_F values. The ratios of hR_F values for the TLC and HPTLC grades as a function of the hR_F values are represented in Fig. 14. On average for the two grades, with a concentrating zone the hR_F value of about 30 is *ca.* 10% higher than that for the uniform layers. A certain tendency is detectable in the direction of

TABLE IX

hR_F VALUES AND hR_F VALUE RATIOS FOR SILICA GEL 60 PRE-COATED TLC PLATES AND SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE

Mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_f = 100 mm.

		4 µg	8 µg	12 µg	16 µg	20 µg	Mean Value	Relation between TLC-Silica gel with Conc.Zone and TLC-Silica gel Mean Value
hR_f	V I							
	TLC-Silica gel	73,6	73,7	73,5	73,0	72,9	73,3	1,02
	TLC-Silica gel with Conc.Zone	74,9	74,3	74,5	74,6	74,6	74,6	
hR_f	V II							
	TLC-Silica gel	48,0	48,0	47,6	46,8	46,4	47,4	1,05
	TLC-Silica gel with Conc.Zone	50,3	49,7	49,8	49,8	49,6	49,9	
hR_f	Gr							
	TLC-Silica gel	38,8	38,8	38,3	37,5	37,1	38,1	1,09
	TLC-Silica gel with Conc.Zone	41,8	41,3	41,6	41,6	41,4	41,5	
hR_f	B							
	TLC-Silica gel	15,1	14,9	14,6	14,0	13,9	14,5	1,14
	TLC-Silica gel with Conc.Zone	17,5	16,6	16,5	16,4	15,8	16,6	
hR_f	R							
	TLC-Silica gel	9,4	9,1	9,0	8,6	8,6	8,9	1,09
	TLC-Silica gel	10,3	9,6	9,7	9,6	9,1	9,7	

the conditions of circular chromatography. The higher, *i.e.*, more favourable kappa value of the HPTLC plate with the concentrating zone can be explained by the comparatively high velocity of the migrating substances in the concentrating zone (Table XI).

The separation performances of the silica gel 60 pre-coated HPTLC plate with a concentrating zone in comparison with that of the silica gel 60 pre-coated TLC plate with a concentrating zone as a function of the amount applied for a run of 50 mm are represented in Table XII and Fig. 15. The separation numbers for a wide range of amounts applied (between 10 ng and 20 µg) are about 40% higher for the pre-coated HPTLC plate with a concentrating zone.

Table XIII gives chromatographic data obtained in various runs for both grades of layer with a concentrating zone. The base widths of the substance peaks, w_x, as a function of the distance run by the substance, z_x, are compared in Fig. 16, and the separation numbers, SN, as a function of the distance run by the solvent, z_f, in Fig. 17. A base width of a peak of, *e.g.*, 4 mm is achieved with a substance run of 15 mm on the TLC plate with a concentrating zone, but on the HPTLC plate with a concentrating zone very much more favourably with a run of 40 mm (Fig. 16). The same chromatographic performance with a separation number of, for example, 12 is

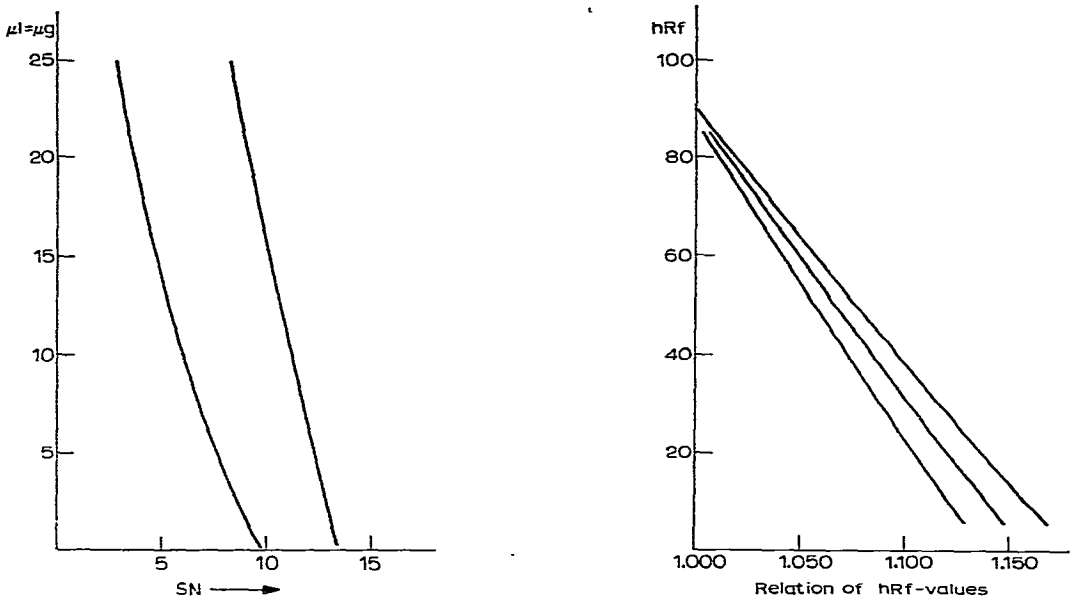

Fig. 13. Separation numbers *versus* amounts applied on silica gel 60 pre-coated HPTLC plates (left) and silica gel 60 pre-coated HPTLC plates with a concentrating zone (right); for experimental conditions, see Table X.

Fig. 14. Ratios of hR_F values *versus* hR_F values for layers with a concentrating zone and layers without a concentrating zone; TLC grades (left), HPTLC grades (right), mean values (centre); for experimental conditions, see Tables IX and X.

attained on the pre-coated TLC plate with a concentrating zone with a solvent run of 80 mm, but on the pre-coated HPTLC plate with a concentrating zone with a run of 45 mm (Fig. 17). With the same solvent runs, the kappa values, which represent the flow-rates, are the same for both plate grades with concentrating zones (Table XIII).

Fig. 18 compares chromatograms of the same separations with different amounts of substance on silica gel 60 pre-coated TLC plates and silica gel 60 pre-coated TLC plates with a concentrating zone with a z_f value of 100 mm and on silica gel 60 pre-coated HPTLC plates and silica gel 60 pre-coated HPTLC plates with a concentrating zone with a z_f value of 50 mm from the start line to the solvent front. Consideration of the second and third columns of Fig. 18 permits a comparison of the plate grades without concentrating zones, and consideration of the first and second or the third and fourth columns permits a comparison of the respective layers without and with a concentrating zone. On consideration of the same layer with increasing amounts applied, a noticeable deterioration is observed for the plates without a concentrating zone, but only a slight deterioration in the efficiency of separation for the plates with a concentrating zone. The not entirely satisfactory quality of separation apparent in the chromatograms obtained with the pre-coated HPTLC plates can be explained by the 10- to 100-fold overloading of the layers in comparison with the amounts of 10–100 nl usually applied on pre-coated HPTLC plates.

TABLE X

SEPARATION NUMBERS WITH VARIOUS AMOUNTS APPLIED FOR SILICA GEL 60 PRE-COATED HPTLC PLATES AND SILICA GEL 60 PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_f = 50 mm.

Amount applied µg	Separation Number HPTLC – Silica gel	HPTLC – Silica gel with Conc.Zone	Relation of the Separation Numbers between HPTLC-Silica gel with Conc.Zone and HPTLC-Silica gel
0,01	9,78	13,40	1,37
0,1	9,74	13,38	1,37
1	9,32	13,15	1,41
2	8,88	12,89	1,45
3	8,45	12,65	1,50
4	8,05	12,41	1,54
5	7,67	12,17	1,59
6	7,31	11,94	1,63
7	6,96	11,71	1,68
8	6,63	11,49	1,73
9	6,31	11,26	1,78
10	6,01	11,05	1,84
15	4,72	10,04	2,13
20	3,60	9,11	2,47
25	2,90	8,28	2,86
50	0,86	5,11	5,94

SPECIAL CHROMATOGRAPHIC BEHAVIOUR OF THE CONCENTRATING ZONE

When plates with a concentrating zone are used, it is generally possible to employ a solvent or solvent mixture as for chromatography on conventional layers. If, however, certain substance components are retained in the starting spot or in the concentrating zone, there may be two reasons. This effect may firstly indicate insolubility or at least a low solubility of the constituents in the solvent concerned. This effect can usually be overcome by changing the composition of the solvent while retaining the overall polarity.

The higher the solubility of the substance in the solvent, the narrower is the concentrating of the substance in the interface and the more favourable is the starting situation for chromatography. For example, with lipophilic dyestuffs (see Fig. 19a–k), marked differences are detectable. The upper section shows the single development up to the interface, and the lower section the course of the chromatography in the

TABLE XI

hR_F VALUES, hR_F VALUE RATIOS AND KAPPA VALUES FOR SILICA GEL 60 PRE-COATED HPTLC PLATES AND SILICA GEL 60 PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_f = 50 mm.

		2 µg	4 µg	8 µg	Mean Value	Relation between HPTLC-Silica gel with Conc.Zone and HPTLC-Silica gel Mean Value
hR_f	V I					
	HPTLC-Silica gel	72,0	71,3	71,4	71,6	1,03
	HPTLC-Silica gel with Conc.Zone	74,2	73,7	73,5	73,8	
hR_f	V II					
	HPTLC-Silica gel	46,6	46,0	46,3	46,3	1,09
	HPTLC-Silica gel with Conc.Zone	51,2	50,3	49,6	50,4	
hR_f	Gr					
	HPTLC-Silica gel	37,7	37,3	37,7	37,6	1,11
	HPTLC-Silica gel with Conc.Zone	42,3	41,6	41,1	41,7	
hR_f	B					
	HPTLC-Silica gel	15,0	14,7	14,8	14,8	1,11
	HPTLC-Silica gel with Conc.Zone	17,9	16,3	15,1	16,4	
hR_f	R					
	HPTLC-Silica gel	9,0	8,9	9,0	9,0	1,19
	HPTLC-Silica gel with Conc.Zone	11,9	10,6	9,5	10,7	
$\varkappa$						
	HPTLC-Silica gel				3,13	1,28
	HPTLC-Silica gel with Conc.Zone				$4,0^{x}$	

x from interface

solvent concerned. Chloroform (Fig. 19d), acetonitrile (Fig. 19f), ethyl acetate (Fig. 19g) and acetone (Fig. 19h) are particularly suitable for concentrating purposes. *n*-Hexane (Fig. 19a) is unsuitable. The remaining solvents can be used, as they gradually dissolve the substances during the longer passage of the solvent and apparently bring them to the chromatographic process without an unfavourable influence on the separation performance.

The reason why certain substances are completely or partially retained in the concentrating zone and do not migrate to the interface lies in the chromatographic properties of these substances in the concentrating zone in the solvent used. Such substances are usually inorganic and sometimes organic salts, or very polar and usually low-molecular-weight substances with alkyl, carbonyl or nitrile groups, *e.g.*, certain alcohols and sugars. When hydrophilic solvents are used, partition chroma-

TABLE XII

SEPARATION NUMBERS FOR VARIOUS AMOUNTS APPLIED ON SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE AND SILICA GEL 60 PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_f = 50 mm.

Amount applied µg	Separation Number TLC – Silica gel with Conc.Zone	HPTLC – Silica gel with Conc.Zone	Relation of the Separation Numbers between HPTLC-Silica gel with Conc.Zone and TLC-Silica gel with Conc.Zone
0,01	9,09	13,16	1,45
0,1	9,07	13,14	1,45
1	8,96	12,93	1,44
2	8,83	12,70	1,44
3	8,70	12,47	1,43
4	8,58	12,25	1,43
5	8,45	12,03	1,42
6	8,33	11,82	1,42
7	8,21	11,61	1,41
8	8,10	11,40	1,41
9	7,98	11,20	1,40
10	7,86	11,00	1,40
15	7,32	10,06	1,37
20	6,81	9,19	1,35
25	6,33	8,40	1,33
50	4,41	5,36	1,21
100	2,14	2,19	1,02

tography can take place even in the very narrow, inert layer of the concentrating zone, so that the substances do not reach the interface, *i.e.*, the starting line for chromatography.

This effect is of advantage if it results in removal of undesirable constituents that interfere in the chromatography; however, it is useless if as a consequence the substance to be determined reaches the actual chromatographic layer only in part or not at all. A change of solvent can be of assistance in this connection. If the cation or anion component of a salt is the moiety to be determined, this moiety into the basic or acidic form must be converted by addition of an alkali or an acid to the solvent (an extremely small amount usually suffices). In such instances it is advisable to select a solvent that is suitable for concentrating purposes by means of a test strip coated with

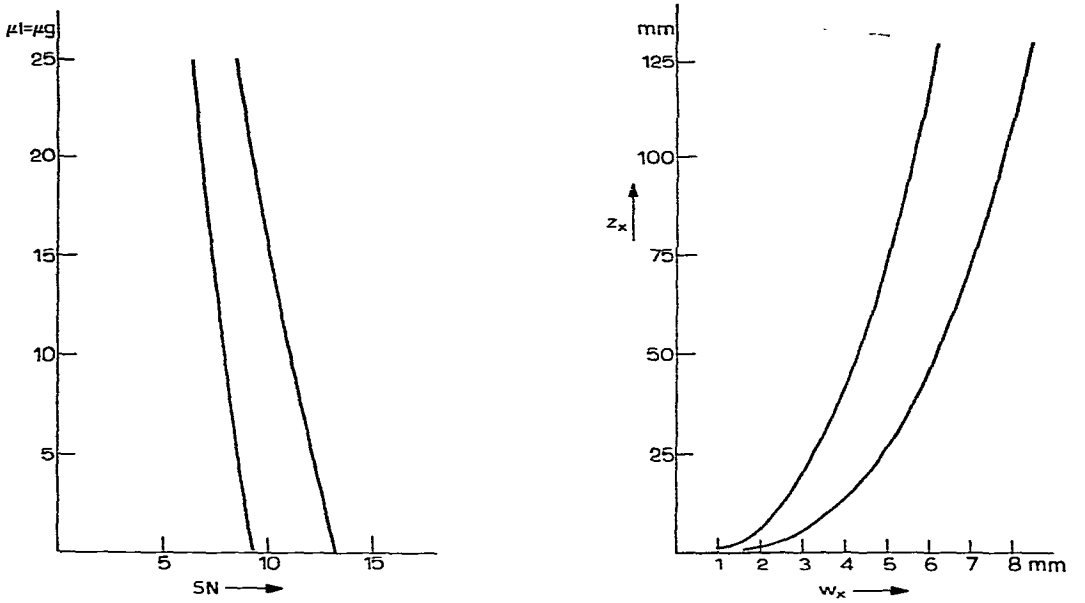

Fig. 15. Separation number *versus* amounts applied on silica gel 60 pre-coated TLC plates with concentrating zone (left) and silica gel 60 pre-coated HPTLC plates with concentrating zone (right); for experimental conditions see Table XII.

Fig. 16. Curves of base widths w_x of the substances *versus* z_x of the substances on silica gel 60 pre-coated TLC plates with a concentrating zone (right) and silica gel 60 pre-coated HPTLC plates with a concentrating zone (left); for experimental conditions, see Table XIII.

the inert material of the concentrating zone. The substances of interest must be entrained with the solvent front.

Further difficulties can arise when the solutions applied to the concentrating zone are too concentrated, with the result that once they have started to dry, a barrier to passage of the solvent arises owing to crystallization or adhesion. In such instances the solution must either be applied in a more diluted form, such drying must be avoided, or the application should be made intentionally to a moistened layer.

SPECIAL PERFORMANCE AND SPECIAL ADVANTAGES

Figs. 20, 21 and 22 show chromatograms of dyestuffs, steroids and amino acids obtained on silica gel 60 pre-coated TLC plates and silica gel 60 pre-coated TLC plates with a concentrating zone. Owing to the formation of lines, the separations are significantly improved over the entire chromatographic region, and the improvement is especially marked in the lower R_F value range and when very large amounts are applied.

Fig. 23 compares separations of lipophilic dyestuffs with low R_F values at various concentrations in layers without and with a concentrating zone. It can be seen that in the separation of substances with very similar R_F values, small amounts of substances with higher R_F values are easier to separate than small amounts of substances with lower R_F values. The improvement in separation when using the plates

TABLE XIII

CHROMATOGRAPHIC DATA FOR VARIOUS VALUES OF z_f ON SILICA GEL 60 PRE-COATED TLC PLATES WITH A CONCENTRATING ZONE AND SILICA GEL PRE-COATED HPTLC PLATES WITH A CONCENTRATING ZONE

Mixture of three lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene.

		Violet				Green				Blue					
	z_f mm	hR_f	z_x mm	w_x mm	H µm	hR_f	z_x mm	w_x mm	H µm	hR_f	z_x mm	w_x mm	H µm	$\varkappa$ mm^2/s	SN
TLC-Silica gel	141,2	75,7	106,9	7,68	38,5	46,2	65,3	7,90	59,6	17,8	25,1	5,63	79,0	5,1 x	15,7
with Conc.Zone	121,2	76,1	92,3	6,78	31,1	45,9	55,7	7,50	63,1	17,0	20,6	5,04	77,3	5,0 x	14,7
	102,1	75,5	77,1	6,50	34,2	45,9	46,9	6,55	57,2	18,4	18,8	4,74	74,9	5,0 x	13,4
	80,5	76,5	61,6	5,45	30,1	46,6	37,6	6,06	61,2	18,1	14,6	4,39	82,5	4,6 x	11,8
	60,5	75,6	45,8	4,62	29,2	45,2	27,4	4,84	53,5	17,0	10,3	3,83	88,9	4,5 x	10,5
	40,4	73,3	29,6	4,26	37,6	42,4	17,1	4,12	62,1	14,3	5,8	2,99	96,7	3,7 x	7,7
	20,5	71,4	14,6	4,02	68,8	39,5	8,1	3,22	80,2	13,8	2,8	1,98	86,8	2,6 x	4,2
HPTLC-Silica gel	69,8	75,0	52,4	4,35	22,5	44,7	31,2	4,11	33,9	17,3	12,1	3,06	48,5	4,4 x	14,1
with Conc.Zone	61,0	76,0	46,4	3,85	20,0	48,5	29,6	3,73	29,6	20,3	12,3	2,93	43,5	4,2 x	13,6
	40,3	76,7	31,0	2,98	17,9	46,3	18,7	3,00	30,0	19,0	7,7	2,18	38,8	3,5 x	11,4
	20,0	77,1	15,4	2,20	19,6	45,7	9,1	1,96	26,1	19,1	3,8	1,47	35,4	2,7 x	7,9

x from interface

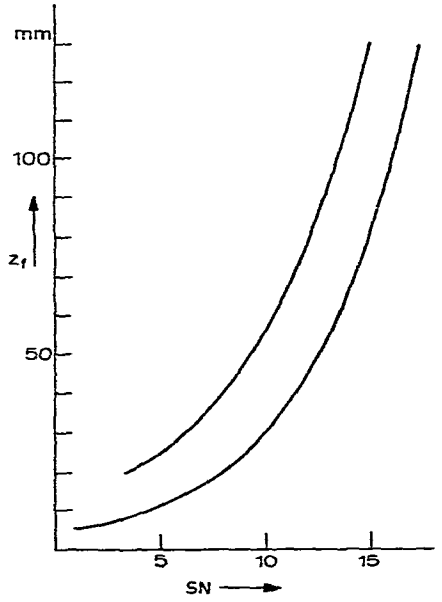

Fig. 17. Separation numbers *versus* z_f of the solvent on silica gel 60 pre-coated TLC plates with a concentrating zone (left) and silica gel 60 pre-coated HPTLC plates with a concentrating zone (right); for experimental conditions, see Table XIII.

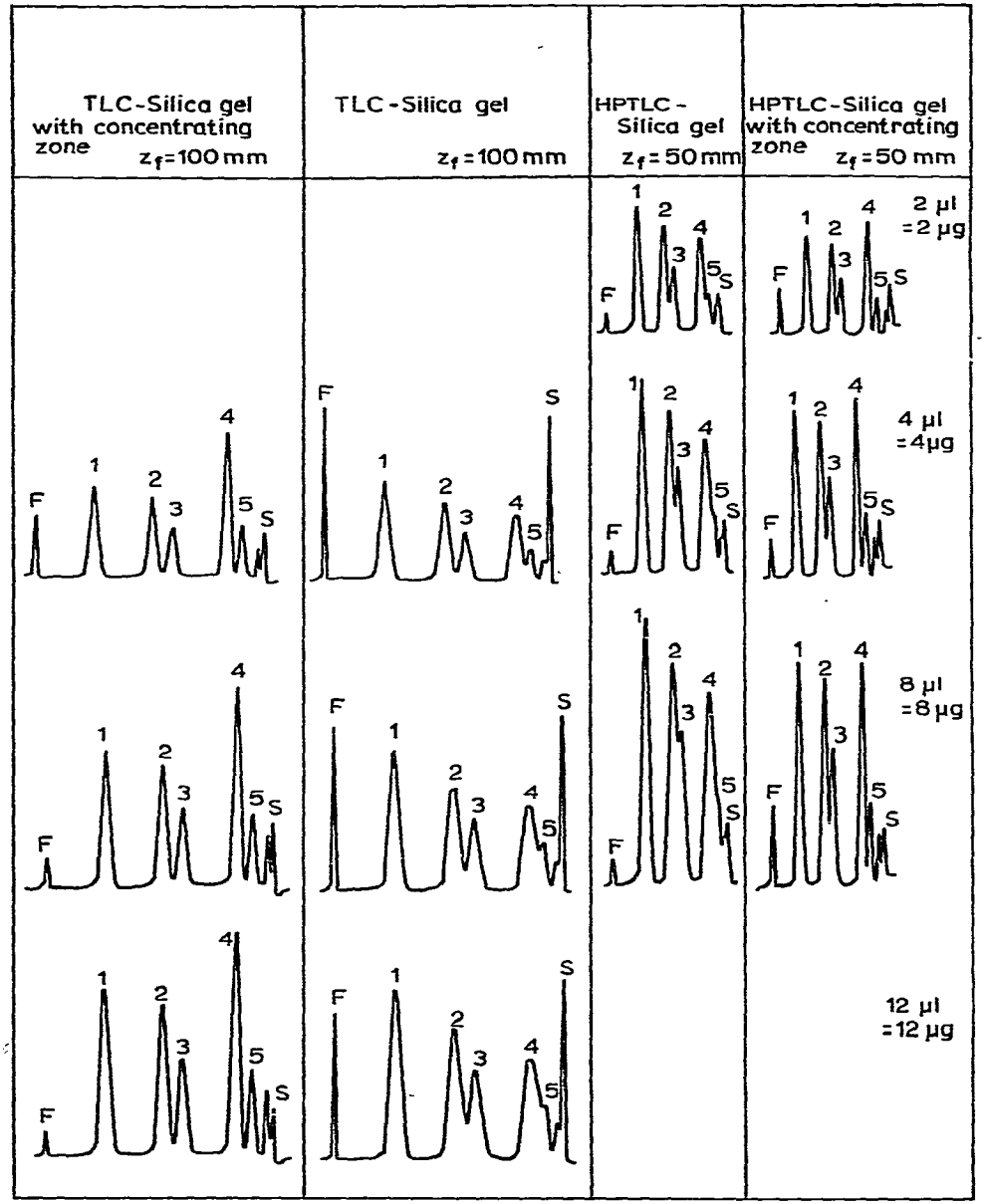

Fig. 18. Chromatograms for various amounts applied on silica gel 60 pre-coated TLC plates (z_f = 100 mm) and on silica gel 60 pre-coated HPTLC plates (z_f = 50 mm) without and with concentrating zones; mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, 586 nm.

with a concentrating zone can clearly be seen (Fig. 23b is better than Fig. 23a and Fig. 23d is better than Fig. 23c).

The concentrating effect permits the immediate application of spots or larger areas, without the need for the usual prior enrichment process, and these merge in a

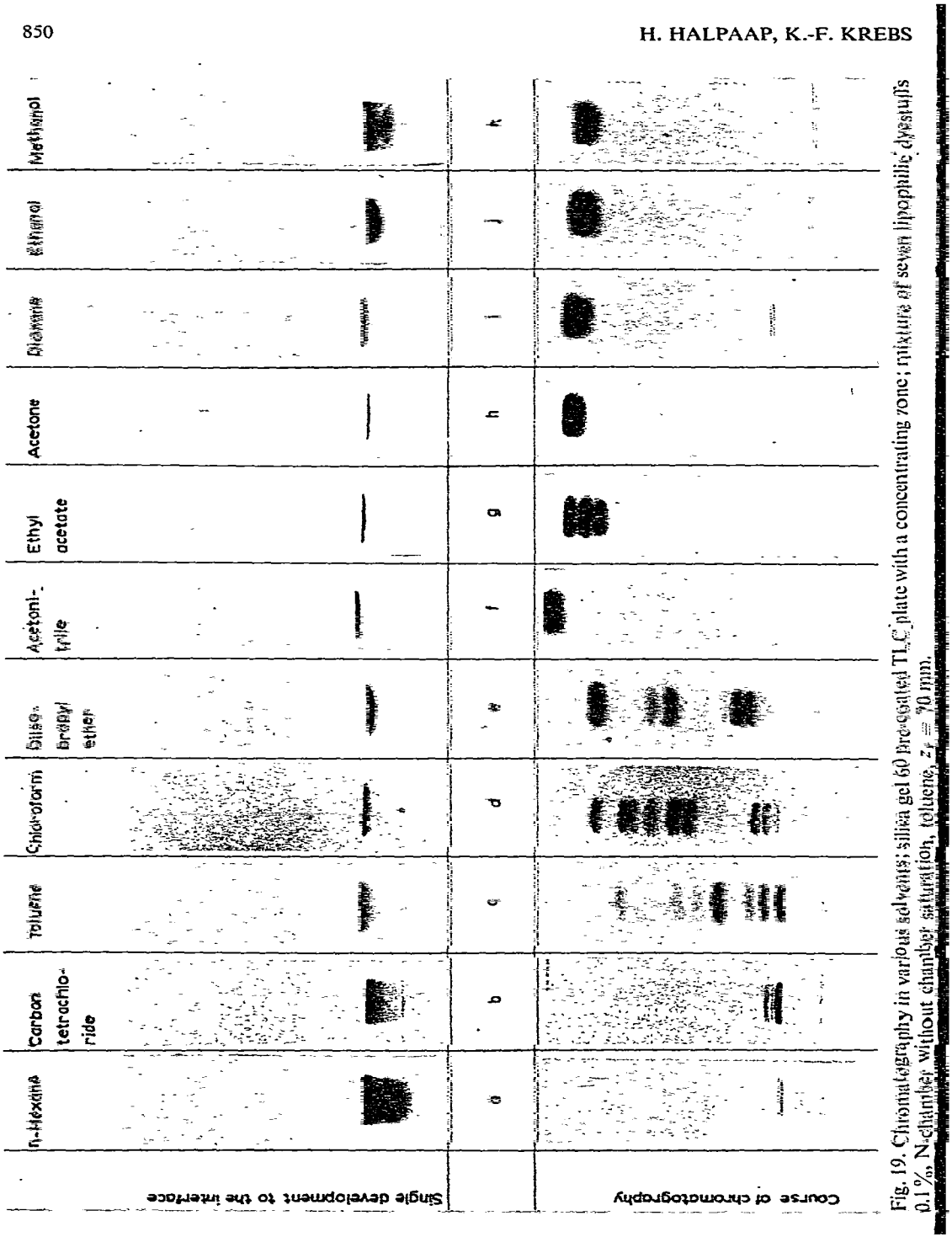

Fig. 19. Chromatography in various solvents; silica gel 60 precoated TLC plate with a concentrating zone; mixture of seven lipophilic dyestuffs 0.1%, N-chamber without chamber saturation, toluene, z_F = 70 mm.

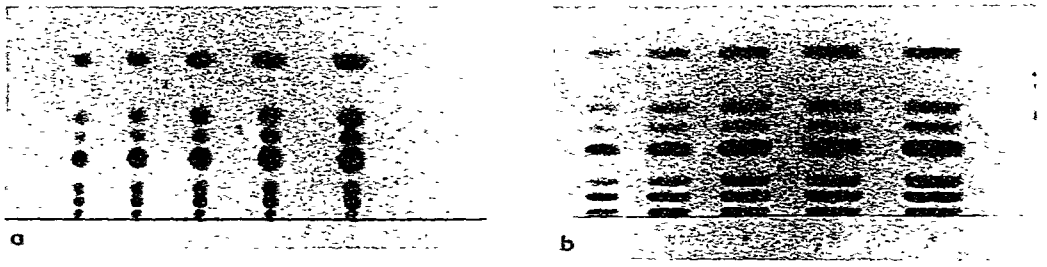

Fig. 20. Chromatograms on (a) silica gel 60 pre-coated TLC plates and (b) silica gel 60 pre-coated TLC plates with a concentrating zone of a mixture of seven lipophilic dyestuffs; amounts applied (from left to right): 4, 8, 12, 16, and 20 μl of the 0.1% solution; N-chamber without chamber saturation, toluene, z_f = 100 mm.

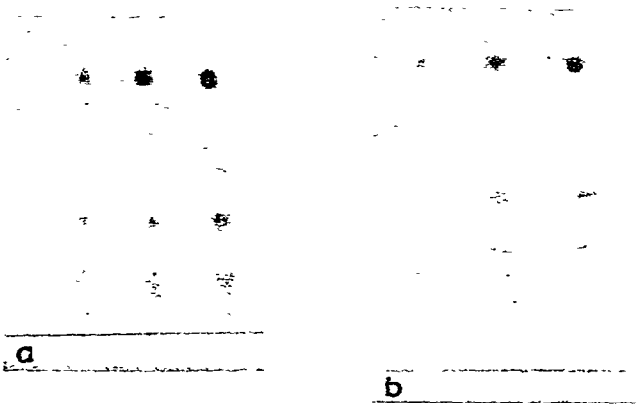

Fig. 21. Chromatograms on (a) silica gel 60 pre-coated TLC plates and (b) silica gel 60 pre-coated TLC plates with a concentrating zone of a mixture of steroids (cholesterol stearate, chlormadinone acetate, cholesterol, epitestosterone, pregnanediol, 11-deoxy-17-hydroxycorticosterone and corticosterone), amounts applied (from left to right) 0.75, 2, and 4 μl of the 0.1% solution per steroid, N-chamber with chamber saturation, solvent: chloroform–methanol (95:5), z_f = 100 mm, immersion in 5% perchloric acid in methanol, 5 min, 120°, UV detection at 366 nm.

Fig. 22. Chromatograms on (a) silica gel 60 pre-coated TLC plates and (b) silica gel 60 pre-coated TLC plates with a concentrating zone of a mixture of amino acids (L-leucine, DL-valine, α-aminobutyric acid, α-alanine, DL-threonine, glycine, glutamine and arginine), amounts applied (from left to right) 0.75, 2, 4, 6, and 8 μl of a 0.02% solution per amino acid, N-chamber without chamber saturation, solvent: propanol–water (80:20), z_f = 100 mm, 2 min, 120°, ninhydrin.

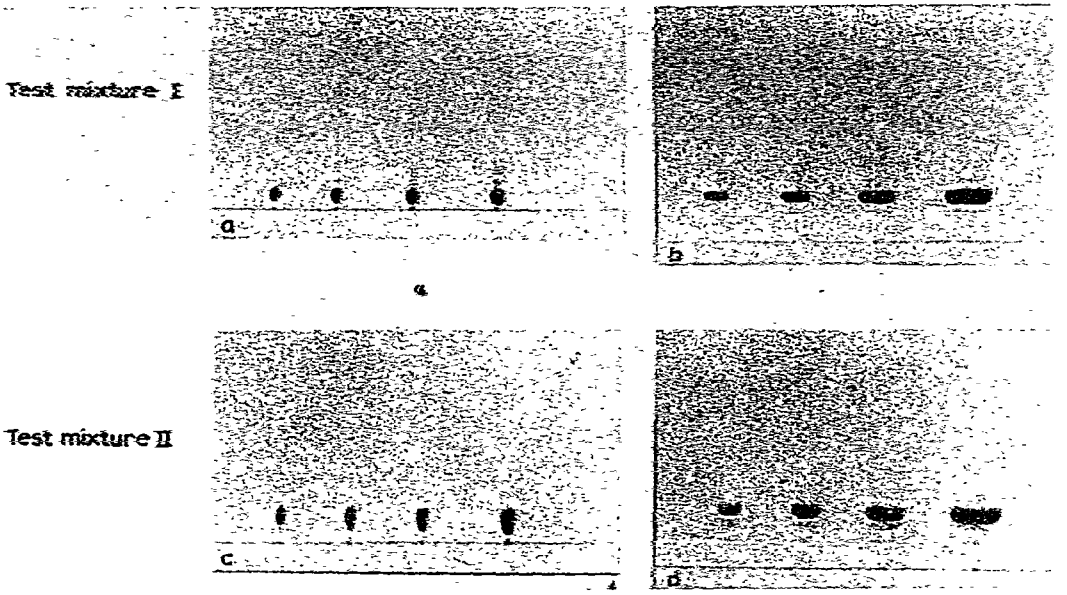

Fig. 23. Chromatograms on (a and c) silica gel 60 pre-coated TLC plates and (b and d) silica gel 60 pre-coated TLC plates with a concentrating zone of substances in varying concentrations. Test mixture I: 0.005% Blue VIF Organol (higher R_F value) and 0.5% Ceres red G (lower R_F value). Test mixture II: 0.5% Blue VIF Organol (higher R_F value) and 0.005% Ceres red G (lower R_F value). Amounts applied (from left to right): 2, 4, 8, and 16 μl (equivalent to 0.1/10, 0.2/20, 0.4/40, and 0.8/80 μg); N-chamber without chamber saturation, toluene, z_f = 100 mm.

continuous flow into the chromatographic layer. Losses cannot occur with this technique, and the possibility of decomposition, which cannot be completely avoided even with the most careful concentration, is virtually excluded in the inert layer. The sensitivity necessary for the detection of very small amounts of substances is increased automatically by the concentrating effect or even provided at all.

A further advantage of the technique is that salts or very polar, non-salt-like compounds are retarded in the preliminary layer of the concentrating zone. This often makes it possible to effect for the first time a chromatographic separation of the substances of interest without interference by such compounds. The sample clean-up that is necessary particularly in biochemistry is carried out almost automatically in this layer before chromatography. Beesley[7] in 1972 drew attention to the possibility of the direct application of blood, serum or urine to a pre-adsorbent layer.

By direct application of urine to the concentrating zone by immersion, subsequent drying and further application of a spot of a mixture of reference substances at two sites in the concentrating zone, we were able in several instances immediately after chromatography to detect new urinary metabolites, classify them and determine them. This should lead to many applications in biochemistry, metabolite research, pharmaceutical quality control, food chemistry, the chemistry of natural substances and other fields.

The possibility of the virtually problem-free application of larger amounts of substances considerably extends the efficacy of the chromatographic layer. In short runs on the HPTLC layer in particular, separations in the nanolitre range formerly

required a laborious application. On an HPTLC plate with a concentrating zone equally good separations can be attained with application in the microlitre range.

FUTURE DEVELOPMENTS

The method described here for TLC permitting attainment of a high dosage quality crucial to successful chromatography by formation of an extremely sharp interface between a special inert sorbent and the actual chromatographic sorbent is of general significance. This method should gradually become established as a useful thin-layer chromatographic technique because of its improved separation performance and sensitivity, and also because of the elimination of various problems and the saving of time in application. In preparative-layer chromatography, with its aim of isolating substances for preparative purposes, the technique is important because of its high application capacity with simultaneous high separation performance.

The inert synthetic porous silica sorbent in an appropriate arrangement can also be used in preparative and analytical column chromatography. The first results achieved in our laboratories are, as expected, analogous to those in thin-layer chromatography.

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