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STATIONARY PHASES FOR HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The characteristics of commercially available high-performance thin-layer chromatographic quality thin-layer plates pre-coated with silica gel, cellulose, chemically bonded phases or polyamide are discussed and compared with those of pre-coated layers used in conventional thin-layer chromatography.

INTRODUCTION

In recent years, high-performance thin-layer chromatography (HPTLC) has rapidly gained widespread acceptance. In the literature, the importance of aspects such as improved dosage methods, optimal techniques for layer conditioning and feeding the mobile phase and the use of suitable data acquisition techniques has repeatedly been stressed. Most attention, however, has been devoted to the development of suitable stationary phase materials.

In this paper, information is presented on the characteristics of the pre-coated stationary phases for HPTLC that are commercially available. In addition, experimental results obtained with several of the newer types of material are reported.

EXPERIMENTAL

Commercially available pre-coated thin-layer plates used in this study included HPTLC and conventional TLC silica gel plates from Merck (Darmstadt, G.F.R.; product nos. 5628 and 5715, respectively) and Macherey, Nagel & Co. (Düren, G.F.R.; product nos. Sil-20 UV₂₅₄ and Sil G-25 HR/UV₂₅₄, respectively), HPTLC silica gel plates from Schleicher & Schüll (Dassel, G.F.R.; product no. G 1570/LS 254), HPTLC and conventional TLC cellulose plates from Merck (product nos. 5787 and 5716, respectively), plates coated with LiChrosorb RP-18 (Merck; product no. 13724), KC₁₈ thin-layer plates from Whatman (Springfield Mill, Great Britain; product no. 4803600), plates silanized 50, 75 or 100% with a C₁₈ alkylsilane (Macherey, Nagel & Co.; product nos. Sil C 18-50, -75 and -100 UV₂₅₄, respectively, and designated MN50, MN75 and MN100 in this paper) and plates coated with HPTLC or

conventional TLC polyamide (Macherey, Nagel & Co.; designation Wang-Polyamid-6 and Polygram Polyamid-6 UV₂₅₄, respectively). Further details on these and other types of thin-layer plates are given later.

All solvents, model compounds and further chemicals used were of normal analytical-reagent grade.

Details of the chromatographic procedures routinely used in our laboratory have been published recently¹.

RESULTS AND DISCUSSION

Silica gel

Silica gel thin-layer plates were the first pre-coated slides to be offered for sale in HPTLC quality. To-day, Merck sells a complete range of pre-coated plates, in different sizes, with or without an added fluorescent indicator (F₂₅₄), and with or without a concentration zone. Also, plates are available that have an aluminium instead of a glass backing. Less varied supplies of pre-coated silica gel plates for HPTLC are marketed by Schleicher & Schüll, Macherey, Nagel & Co. and Whatman; again, plates with or without a fluorescent indicator and, occasionally, plates with a concentration zone are offered. HPTLC plates, whether with a silica gel or another type of coating, are frequently termed "Nano Plates" on account of the recommended 10–200-nl dosage volume. It is interesting that, confusingly, in several early papers on HPTLC authors stated they carried out separations on "Nano Plates" without specifying the type of stationary phase material used. However, because at that time only HPTLC plates pre-coated with silica gel were commercially available, it is reasonable to conclude that these were the chromatoplates actually used.

A serious disadvantage of all three types of silica gel HPTLC plates studied (Merck, Schleicher & Schüll, Macherey, Nagel & Co.) is the relatively long development time compared with that observed with conventional TLC plates (Table I; similar results have been obtained with other mobile phases). Using the well-known relationship

$$z_f^2 = kt \quad (1)$$

where z_f is the distance of the run and t is the time of the run, one can calculate that the velocity constant, k , of HPTLC plates is about 40% smaller than on conventional TLC plates. This conclusion is in fair agreement with literature data².

TABLE I

DEPENDENCE OF TIME OF RUN ON TYPE OF SILICA GEL USED IN (HP)TLC FOR TWO SOLVENTS

Stationary phase	$t_{5\text{-cm run}}$ (min)		$100R_F/R_{F,Mc HP}^*$
	Toluene	Chloroform	
Merck TLC	6.5	6	98–102
Macherey, Nagel & Co. TLC	6	6	102–106
Merck HPTLC	10.5	11	100
Macherey, Nagel & Co. HPTLC	10	11	99–101
Schleicher & Schüll HPTLC	10.5	10	96–99

* $R_{F,Mc HP}$ denotes R_F values on Merck HPTLC plates; results are based on data for 10–15 compounds.

In an obvious attempt to remedy this disadvantage of silica gel HPTLC plates, Merck has recently announced the production of a new series of plates, which will gradually replace the older type. The new plates display² a higher k value than the older plates, *e.g.*, 4.5 vs. 3.8 mm²/sec for toluene as solvent. The velocity constant is, however, still lower than that recorded for conventional TLC silica gel (5.4 mm²/sec). This agrees with our experimental data: a 5-cm run with toluene took about 8 min on a "new" HPTLC plate compared with 10 min on an "old" HPTLC plate, and 6 min on a conventional TLC plate (*cf.*, Table I).

Another noteworthy aspect is the relative contribution of longitudinal molecular diffusion and mass transfer (plus the quality of the packing) to band broadening in (HP)TLC. On the basis of extensive discussions, Guiochon and co-workers^{3,4} have concluded that HPTLC on silica gel, with its relatively low mobile phase velocities, is carried out under conditions such that molecular diffusion largely determines the spot length, *i.e.*, the standard deviation of the spot peak, σ_x , and, therefore, the average plate height, H_{av} , defined as

$$H_{av} = \sigma_x^2/z_x \quad (2)$$

where z_x is the distance migrated by the spot. Conventional TLC, on the other hand, has been shown to work under experimental conditions such that resistance to mass transfer controls the spot length. According to Guiochon and co-workers, as a consequence H_{av} will increase very rapidly with increasing length of run if very fine silica gel particles ($d_p < ca. 7 \mu\text{m}$, generally stated to be typical for HPTLC; however, see below) are used. With the larger (10–15 μm) conventional TLC type of particles, on the other hand, the increase in H_{av} with increasing length of run will be much slower. If this is true, then the low plate heights said to be commonly achieved in HPTLC can be obtained only if the diffusion coefficient, D , of the solute is very small, *i.e.*, if the solute molecules are relatively large. In other words, to quote Guiochon and Siouffi⁴, it may be of some significance "... that most demonstrations of the performance of HPTLC are made using mixtures of dyes which have rather large molecular weight".

Apart from some data recorded in ref. 4, no experimental verification of the above conclusions has been published. We therefore carried out the TLC of two chloroanilines and two, presumably high-molecular-volume, dyestuffs in the system silica gel–toluene. Development of the chromatograms in HPTLC and conventional TLC was done under identical conditions. From Figs. 1 and 2 one can conclude that the above hypotheses are confirmed. With the two relatively low-molecular-volume and, therefore, rapidly diffusing chloroanilines, the plate height in HPTLC is seen to reach its minimum value at a small z_f value and to increase very rapidly with increasing length of run; a similar steep increase is absent in conventional TLC. With the dyestuffs, which probably will have small D values, the curves in TLC and HPTLC are very similar. These experiments were carried out using "old" HPTLC plates; no data have as yet been collected for the "new" plates.

Two more remarks should be made. (1) The H vs. z_f curves in Figs. 1 and 2 display minimum plate heights, H_{min} , which are distinctly larger than are those sometimes cited in the literature, *e.g.*, 30 and less than 12 μm for conventional TLC and HPTLC, respectively, in a much quoted table reported in ref. 5 (although inspection

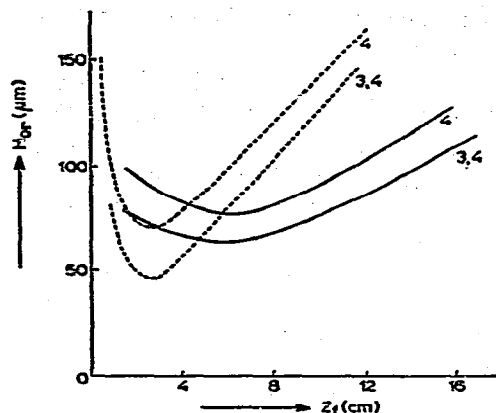


Fig. 1. H vs. z_f curves for 4-chloroaniline (R_F 0.20) and 3,4-dichloroaniline (R_F 0.35) in the system silica gel-toluene using HPTLC (broken lines) and TLC (solid lines) silica gel. Sample volume, 200 nl. The experimental H_{av} values have been corrected for the plate-height contribution due to original spot length, using the relationship $H_{or} = H_{av} - \sigma_{or}^2/z_x$.

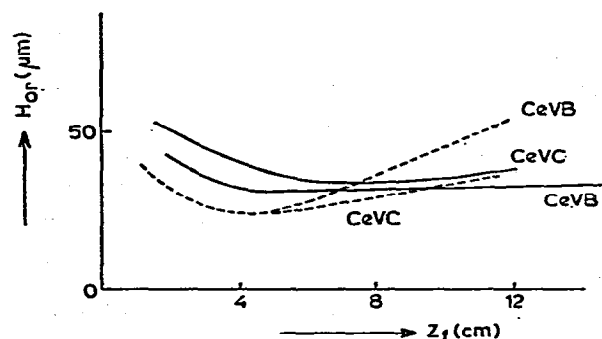


Fig. 2. H vs. z_f curves for two Ceres Violet constituents (R_F 0.60 and 0.85) in the system silica gel-toluene using HPTLC (broken lines) and TLC (solid lines) silica gel. Sample volume, 200 nl. Other details as in Fig. 1.

of experimental curves in refs. 2 and 6 suggests values of about 30 and 20 μm , respectively, for comparable conditions). Presumably, this divergence can be partly explained* by the fact that some authors (see, e.g., refs. 7 and 8) present, instead of H_{av} (or H_{or} ; cf., legend to Fig. 1), so-called H_{50} data calculated by means of the equation

$$H_{50} = (R_F/0.50)H_{av} \quad (3)$$

where R_F is the R_F value of the model compound. Self-evidently, this mode of calculation considerably affects the H values for compounds that display relatively

* According to information recently received from Dr. H. E. Hauck (Merck), the use of an even slightly imperfect spotting procedure can easily adversely influence spot sizes and hence plate heights.

high retentions, such as the chloroanilines in Fig. 1. (2) The differences observed by us between H_{min} in HPTLC and conventional TLC are rather small, which suggests that the particle size of the HPTLC- and TLC-type silica gels do not differ substantially. In the literature, values such as 7 vs. 9 (ref. 9), 7–8 vs. 10–11 (ref. 10) and 5 vs. 11 (ref. 4) μm have been reported; from data quoted by Kaiser¹¹, a value of about 7–10 μm for HPTLC silica gel can be calculated. The technical bulletin issued by Merck¹² on the new developments incorporated in their HPTLC layers, next to the use of a narrower particle size classification of the silica gel and optimized layer thickness and quality of layer packing, merely mentions that the particle size classification is below the range of that of the usual TLC pre-coated plates. Macherey, Nagel & Co.¹³ state that they use the same silica gel for HPTLC as they do for TLC but, for HPTLC, the material "... is very finely fractionated (mean particle size 5–10 μm)". In summary, the particle size difference between HPTLC and conventional TLC silica gel may be smaller than is often assumed.

Cellulose

HPTLC plates pre-coated with cellulose are marketed by Merck. According to our experience, which is based on the separation of four model systems (*cf.*, Table II), similar spot shapes and R_F values (difference 0.00–0.02 R_F unit) are obtained in HPTLC and conventional TLC. More important, the time of development on the HPTLC plates is much smaller than on the TLC plates. Data for two solvent mixtures are presented in Table III; similar results were obtained with various other acidic,

TABLE II
MODEL SYSTEMS USED FOR COMPARISON OF HPTLC- AND TLC-TYPE CELLULOSE

Mobile phase	Compounds separated*
Acetone–6 <i>N</i> HCl (4:1)	Ni, Mn, Co, Cu, Fe
Methanol–6 <i>N</i> HCl (4:1)	Ba, Sr, Ca, Mg
Chloroform–glacial acetic acid–water (7:6:1)	Tannin, gallic acid, pyrogallol, pyrocatechol
<i>n</i> -Butanol–glacial acetic acid–water (4:1:1)	Glycine, alanine, valine

* In order of increasing R_F values.

TABLE III
DEPENDENCE OF TIME OF RUN ON TYPE OF CELLULOSE USED FOR TWO MOBILE PHASES

System: TLC- or HPTLC-type cellulose (Merck) with (I) acetone–6 *N* HCl (9:1) or (II) *n*-butanol–glacial acetic acid–water (4:1:1) as mobile phase.

Distance (mm)	Time of run (min)			
	I		II	
	TLC	HPTLC	TLC	HPTLC
30	3	2	19	12
45	7	4	36	25
60	12	7	60	35
75	16	11	80	50
90	25	16	120	80

neutral and basic mobile phases. Obviously, k_{HPTLC} is about 50% larger than is k_{TLC} . The advantage of the decrease in time of analysis is not offset by a decrease in performance: H_{av} values were generally found to be distinctly smaller on the HPTLC plates, e.g., 40 μm (HPTLC) vs. 70 μm (TLC) for cobalt(II) at $z_x = 30\text{--}50$ mm. A similar considerable decrease in plate height has been observed by Hauck and Halpaap², who recorded minimum values of 15–20 μm (HPTLC) and 30–35 μm (TLC) with leucine as test compound.

"Inactive" silica

Recently, the use of thin-layer plates pre-coated with a synthetic porous silica (Si 50 000) with an extremely high mean pore diameter of about 5000 nm and a very small internal surface area has been reported². According to the authors, this material, which on the basis of their description probably is similar to the material used to prepare concentration zones on other types of HPTLC plates, should best be compared with Kieselguhr. For D-glucose and D-xylose, which are separated with *n*-propanol–water–25% ammonia solution (80:20:1) as the mobile phase, a reduction in plate height of from 100–140 μm (TLC) to 60–80 μm (HPTLC) for a length of run of 5–8 cm is obtained.

Chemically bonded stationary phases

Thin-layer plates pre-coated with LiChrosorb RP-2, RP-8 or RP-18 and containing a fluorescent indicator (or, in the case of RP-18, without the indicator, but with a concentration zone) are manufactured by Merck. Pre-coated KC₁₈ plates, which contain C₁₈ carbon chains bonded to the silica via Si–O–Si bonds, are supplied by Whatman. With these plates, chemical bonding of the C₁₈ chain is followed by a further reaction with C₂ compounds to obtain a fully covered silica gel surface. The total carbon load of the KC₁₈ layer is 10%, which allows for a high sample capacity. It has been demonstrated¹ that with methanol–water and acetonitrile–water mixtures containing 0–30% of water as the mobile phase, the four types of bonded stationary phase perform equally well; however, the KC₁₈ plates should be favoured because of the relatively short times of run observed for systems involving their use. With mobile phases containing over about 30% of water, the RP-coated plates can no longer be used conveniently owing to the non-wettability of the stationary phase material. No such problem is encountered with the KC₁₈ plates, provided that about 3% of sodium chloride or a similar salt is added to the mobile phase in order to prevent dislodgement of the chemically bonded phase from the glass backing.

Recently, Macherey, Nagel & Co. have marketed¹³ a series of bonded-phase HPTLC plates. The manufacturers prefer the use of a single (C₁₈) alkylsilane with different percentages of silanization over the use of a range, e.g., C₂–C₁₈, of alkylsilanes. HPTLC silica gel was selected as base material, the reactive groups being totally (100%) or partly (75 or 50%) substituted with alkylsilanes. Results for the separation of chloroanilines and polynuclear aromatic hydrocarbons, carried out with a mobile phase containing a relatively small proportion of water, are shown in Fig. 3. As is to be expected, the retention decreases (slightly) with a decreasing percentage of bonded C₁₈ chains. Even with the MN50 material, however, the sorption strength is about equal to that obtained with RP-18-coated layers, while the time of run is considerably shorter. When working with high percentages of water and with 3% of

sodium chloride added, the incompletely substituted MN50 is seen (Fig. 4) to exhibit much better performance than do the 75 and 100% silanized stationary phases. The time of analysis and the separation of the aromatic hydroxy compounds are very similar to those obtained with a Whatman KC₁₈ plate. The presence of a substantial percentage of non-reacted silanol groups on the silica gel surface of MN50 apparently does not affect the chromatographic behaviour of such polar solutes as substituted anilines or aromatic hydroxy compounds. In conclusion, MN50 layers appear to be a valuable addition to the list of chemically bonded stationary phases for HPTLC.

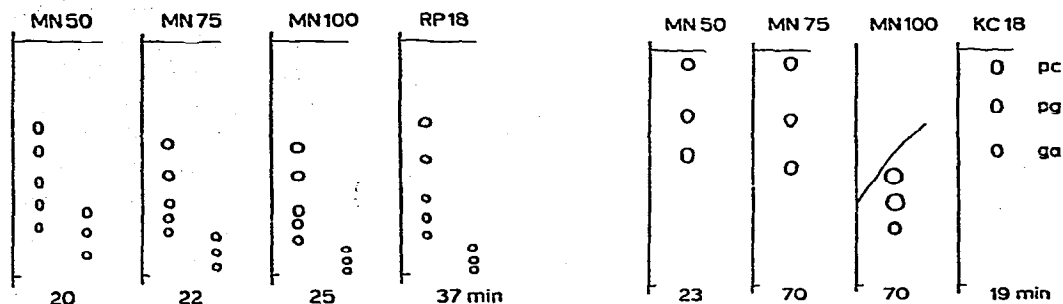


Fig. 3. HPTLC separation of chloroanilines (left) and polynuclear aromatic hydrocarbons (right) on four different chemically bonded stationary phases (for details, see text) using methanol-water (9:1) as mobile phase. Run, 5 cm. Model compounds (in order of decreasing R_F): 4-mono-, 3,4-di-, 2,3,4-tri-, 2,4,6-tri- and 2,3,5,6-tetrachloroaniline; phenanthrene, pyrene and perylene.

Fig. 4. HPTLC separation of aromatic hydroxy compounds on four different chemically bonded stationary phases (for details, see text) using methanol-water (1:1) plus 3% NaCl as mobile phase. Symbols: ga = gallic acid; pg = pyrogallol; and pc = pyrocatechol.

Polyamide

Plastic sheets coated on both sides with polyamide-6, without the use of a binder, are offered¹³ by Macherey, Nagel & Co. under the name "polyamide sheets according to Wang". The separation of series of hydroxy compounds with two mobile phases is illustrated in Fig. 5. Spot size and resolution are seen to be slightly better on the HPTLC than conventional TLC polyamide-6 sheets. However, the time of

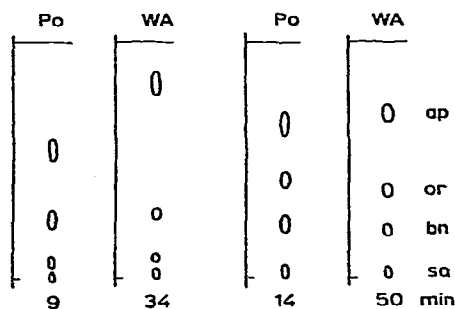


Fig. 5. Separation of salicylic acid (sa), β -naphthol (bn), orcinol (or) and *m*-aminophenol (ap) on polyamide-6 thin layers (Po) and HPTLC polyamide sheets according to Wang (WA), using acetone-water (3:7) and methanol-methyl ethyl ketone-water (1:1:2) as mobile phases.

analysis in HPTLC is 3–4-fold longer, *i.e.*, in an equal time of separation the migration distance in TLC will be about double that in HPTLC. It appears that modification of the process for manufacturing the Wang-type sheets in order to increase considerably their velocity constant would be necessary before they could become useful for analytical purposes.

CONCLUSION

A wide variety of thin-layer plates pre-coated with HPTLC-quality stationary phases are commercially available. As concerns plates pre-coated with silica gel and, even more so, polyamide, the relatively low permeability (small velocity constant) of the HPTLC compared with the conventional TLC plates detracts from their usefulness. With silica gel, this disadvantage has already been partly remedied by the introduction, by Merck, of a newer type of plates that display improved permeability. The use of HPTLC cellulose plates can be recommended on account of their favourable velocity constants and plate heights. The major problem noted so far with chemically bonded reversed phases, *i.e.*, the extremely slow, if any, development of the plates when using mobile phases containing a high proportion of water, can be solved for at least two stationary phase materials by the simple addition of a small amount of sodium chloride to the mobile phase mixture.

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