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From Paper to Planar: 60 Years of Thin Layer Chromatography

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Abstract: The chromatographic techniques using a flat stationary phase and capillary forces to move the mobile phase appeared centuries ago. The paper stationary phase evolved in silica layers on flat surface. The history of 60 years of planar chromatography is told following the evolution of the technique and focusing on the detection modes. The research prototypes are described as well as commercially available equipment. The author concludes by revealing his view of the future of the technique.

Keywords: Planar chromatography, thin layer chromatography, coupling with mass spectrometry

INTRODUCTION

Planar chromatography (PC) is an acronym that appeared in the 1990s. PC is now widely used by scientific journals. Paper chromatography first appeared, followed by thin layer chromatography (TLC), and then appeared the different types of forced flow layer chromatography such as overpressured layer chromatography (OPLC). All these techniques belong to the overall liquid chromatography and more precisely to layer chromatography. In layer chromatography, the column is flat. The purpose of this paper is to give an overview of the history of PC, to remind analysts that this method is very powerful and should never be disregarded, and to forecast the future.

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THE PRECURSORS

An experiment that can be considered as a paper chromatography separation is found in "Historiae Mundii", written at the end of the first century by the Roman writer Pline the younger. In Chapter 24 of his book, he tells how to detect a ferric salt in a pigment. It only needs to spot a drop of the substance onto a papyrus impregnated with oak sap. For centuries linen dyers tried their dyes on sheets of paper or tissues and examined the different colors. Around 1850, Runge, a German pharmacist observed that a drop of pigment mixture deposited onto a paper sheet did not produce a uniform spot but concentric circles of different colors (1). He realized that every colored circle corresponded to a single substance. These colored separations that he called "chemical heralds" were the prelude to the spot tests later developed by Schiff and Feigl. Runge's books are beautiful, as much art as science books. At the same time, Schoenbein, a professor of chemistry in Basel, tried a method to perform quantitation of ozone, that he discovered in 1840. Dipping paper strips in aqueous solutions of starch and iodine, he observed that the two compounds did not migrate to the same level as water. Schoenbein applied the technique to other compounds; capillary chromatography was born. Goppelsroeder established relationships between the chemical nature of the analyzed compounds and the migration rate in the capillarograms (2). At the end of the 19th century, the Dutch biologist Beyerinck (3) observed two rings when a mixture of hydrochloric and sulfuric acids diffused in gelatin; hydrochloric acid moved faster.

In 1944 Consden, Gordon, and Martin (4) separated the amino acids and peptides in wool protein hydrolysates onto a sheet of paper freely suspended in a vapor-tight vessel; the mobile phase was an organic solvent saturated with water. The method combined Tswett's adsorption (the adsorbent is cellulose), the countercurrent distribution, and the Schoenbein's capillary analysis; authors used a descending method. Consden, Gordon, and Martin developed the theoretical aspects with the definition of the R_f (frontal ratio) and showed the two-dimensional capabilities. Three years later Kritchevski and Tiselius (5) introduced the reversed-phase partition chromatography with a nonpolar stationary phase. Paper chromatography was very popular in the 1950s (6) and some analysts remember Whatman paper and the achievements of Munier (7).

Paper chromatography was quickly challenged by thin layer chromatography (TLC). The TLC technique was introduced in 1938 by Izmailov and Shraiber (8) working at Kharkov (USSR). They wanted to separate plant extracts, and observed the similarity between Tswett's adsorption chromatography performed in cylindrical columns and capillarograms (9). They used an alumina-coated microscope slide on which one drop of plant tincture was spotted, followed by dropwise addition of methanol (the solvent they would use in a Tswett's column) onto the spot. They observed several rings under a UV lamp; it is a circular chromatography. They called the

technique "spot chromatography" and the result "ultrachromatograms." Meinhard and Hall (10) used a binding agent to alumina to increase the mechanical resistance of the layer and called the method: "surface chromatography." Thin layer chromatography started its actual development with Egon Stahl (Figure 1) who devised the fabrication of reproducible thin layer coated plates (silicagel according to Stahl, marketed by Merck at Analytica exhibition in Munich in 1958) and standardized the method. He promoted the emerging technique. In 1956 appeared the first paper entitled Thin layer Chromatography (11). Books from Stahl in either German or English languages were best sellers (12, 13). In the USA, Kirchner (14) used strips and was the first to develop quantitative applications. All these developments opened a new era in analytical chemistry.

THE GOLDEN AGE

In the 1950s–1960s HPLC did not exist and column liquid chromatography was performed with glass columns filled with an adsorbent such as silica or

Figure 1. Prof. E. Stahl in his laboratory (courtesy of Merck).

alumina. Gravity was the sole driving force of the liquid and elution times were in nights and days. Furthermore the eluent was collected in tubes that were analyzed one-by-one to detect the solutes. Chemists discovered commercially available standardized plates that allowed the analysis of many samples per run with specific detection by spraying a reagent after development. Chemists dealing with class fractionation and speciation of lipids could separate mixtures of solutes that were unable to be handled by gas chromatography. Table 1 lists the great dates of the evolution of paper chromatography to planar chromatography going through TLC. In 1961 Stahl and coworkers extended the use of TLC to hydrophilic materials that were then the exclusive domain of paper chromatography.

The Simple Old Plates

Thin layer chromatography is a three-phase system (Figure 2). In the simplest mode, solutes are deposited onto the sorbent surface, the plate is placed into a developing chamber (Figure 3) containing at its bottom a small volume of mobile phase that ascents the layer by capillary forces. The distance traveled by the solvent front obeys a quadratic law. The sorbent layer is equivalent to a bundle of interconnected capillaries and the liquid fills first the narrowest capillaries. A liquid enters a capillary because this decreases its free energy. The change in free energy is inversely proportional to the capillary radius. Consequently the solvent front migrates faster than the bulk of the mobile phase (15). In this mode the observed R_f, which is the ratio of distance migrated by the solute over the distance migrated by the mobile phase, is different from the true R_f, which is the ratio of the

Table 1. Historical evolution of chromatographic separation in flat layer

Year	Event
1930	Paper chromatography
1940	Thin layer chromatography (silica and alumina)
1950	Modern TLC and first commercial plates
1960	Spray-on applications and scanning densitometry
1970	High-performance TLC, forced flow TLC (overpressured layer chromatography)
1980	Automatic multiple development, coupling with HPLC, FTIR, ultra-thin TLC
1985	High-performance OPTLC
1988	First issue of *Journal of Planar Chromatography* (Springer Hungarica, Budapest)
1990	Videodensitometry, spherical silica plates
2000	Monolith silica plates (UTLC), coupling with MS-ESI

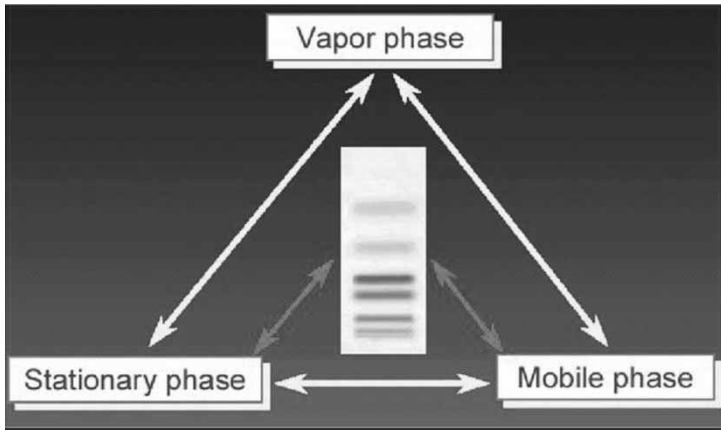

Figure 2. The three phases system in a TLC chamber.

velocities (solute/mobile phase). Furthermore the presence of the gas phase inside the developing chamber (Figure 2) disturbs the velocity of the mobile phase. Evaporation of the low-boiling solvent may occur and capillary rise is longer; conversely, adsorption of vapors from the low-boiling solvent in the tank fills the pores and capillary rise is faster. More often, both adsorption and evaporation occur simultaneously. The phenomenon was investigated and modeled by Guiochon and Siouffi et al. (16) and Geiss (17).

Figure 3. Two classical glass chambers for TLC (photo courtesy of Camag).

Up to the 1970s TLC was a trial-and-error technique with some good practice guidelines. For instance it was often stated that the migration distance of the mobile phase should be at least 10 cm. Fortunately, TLC was (and still is) a unique method to check the chromatographic phenomenon. Soczewinski developed equations for characterizing solvent effects in adsorption TLC and introduced the relation: $R_m = \log (1/R_f - 1)$ (18). L. R. Snyder published extensively (19) on adsorption and TLC retention mechanisms. His book, *Principles of Adsorption Chromatography*, was on the shelf of every chromatographer (20).

In those days, performance was not very high but analysts focused on selectivity; one striking example is the use of silver salt impregnated plates to separate cis–trans isomers. A breakthrough occurred when densitometers were made available in 1969 by manufacturers such as Zeiss, Camag, Kontes, Schoeffel, Joyce-Loebl, or Vitatron. Quantitative determinations became possible. Historically the early densitometers were designed for the scanning of gels. Densitometers are used mostly in reflectance mode. Due to the Kubelka–Munk equation, calibration curves in TLC are nonlinear. Modern densitometers allow multiwavelength scanning, fluorescence measurement, and UV spectra recording (Figure 4).

The First Commercially Elaborated Equipment

The gas phase was still a problem that precluded R_f's reproducibility, and some recipes were advocated. To control the vapor saturation conditions,

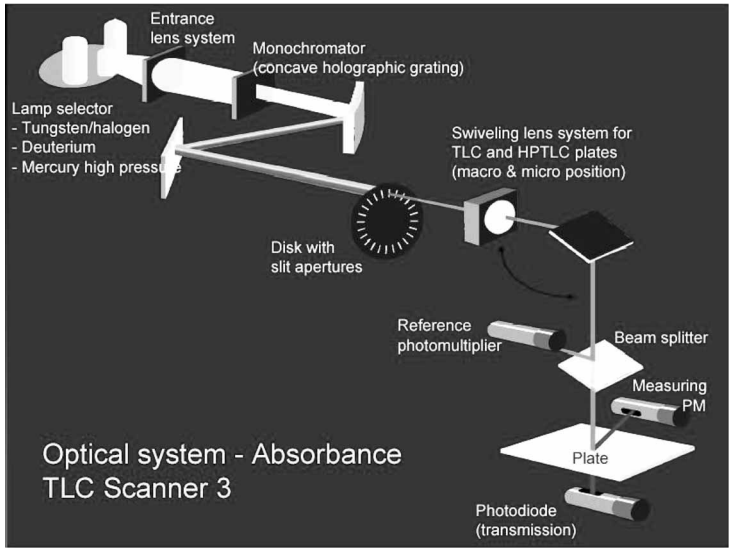

Figure 4. Optical path of a TLC scanner (courtesy of Camag).

Camag introduced in 1970 the Vario KS (for Kammersättigung) (21) to demonstrate that the vapor phase was not necessarily a drawback (22). It was claimed that saturation of the adsorbent allows eliminating all solvent demixing (23). An interesting feature of the Vario KS chamber was the horizontal position of the plate. Gravity is negligible as compared to capillary forces but it enables a better control of the vapor phase and the chromatography starts from the bottom of the plate. Years later, a Polish team also manufactured a horizontal development chamber (Chromdes). Another horizontal chamber that doubled the number of samples from opposite sides of the plate to the middle appeared in the 1980s (Figure 5).

The birth of high pressure liquid chromatography (HPLC) occurred in the mid 1970s. The technique made use of fine particles. At that time Merck introduced the HPTLC plates. The layer of standard TLC plates was made with an 11 μm mean particle diameter, whereas the layer of HPTLC (HP for high performance) plates was made with fine particles of 5 μm mean particle diameter similar to those utilized in HPLC (24). A book dealing with the new possibilities was published (25). Camag introduced the "U" chamber for circular and anticircular development on short distances. Circular TLC allows a lower solvent consumption. Solutes are better resolved in the lower R_f range by circular development than by linear development. Conversely solutes with a

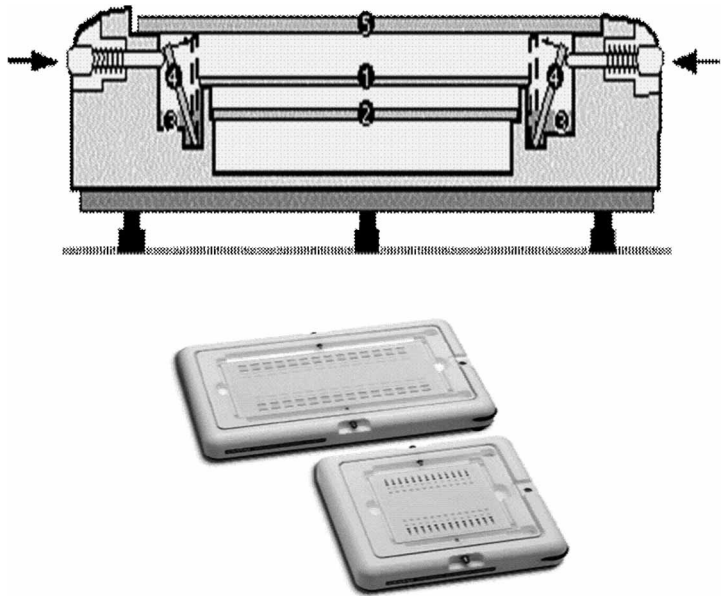

Figure 5. The horizontal chamber scheme (scheme courtesy of Camag). (1) HPTLC plate with silicagel downwards; (2) counter plate for sandwich migration mode; (3) solvent for development; (4) glass strips for starting the capillary contact; (5) closing lid. Bottom: two horizontal chambers still marketed today.

higher R_f range are better resolved by anticircular mode. At that time bare silica plates were only available, which precluded the analysis of very polar solutes; the circular mode was helpful for that purpose.

Guiochon et al. in a series of papers (26, 27), developed the theory of kinetic performances in TLC. They derived equations yielding the height equivalent to a theoretical plate as a function of the development length. The shapes of the curves (Figure 6) are similar to the Van Deemter curves in column chromatography. In these plots the abscissa is the development length since the analyst does not control the velocity of the mobile phase. From the curves it is obvious that a development length of 10 cm was necessary to obtain the best efficiency with the coarser particles that were utilized earlier. The main feature of these curves is that a very short development length is mandatory to get the lowest HETP with fine particles, whereas a longer development length is necessary when particle diameter is increasing.

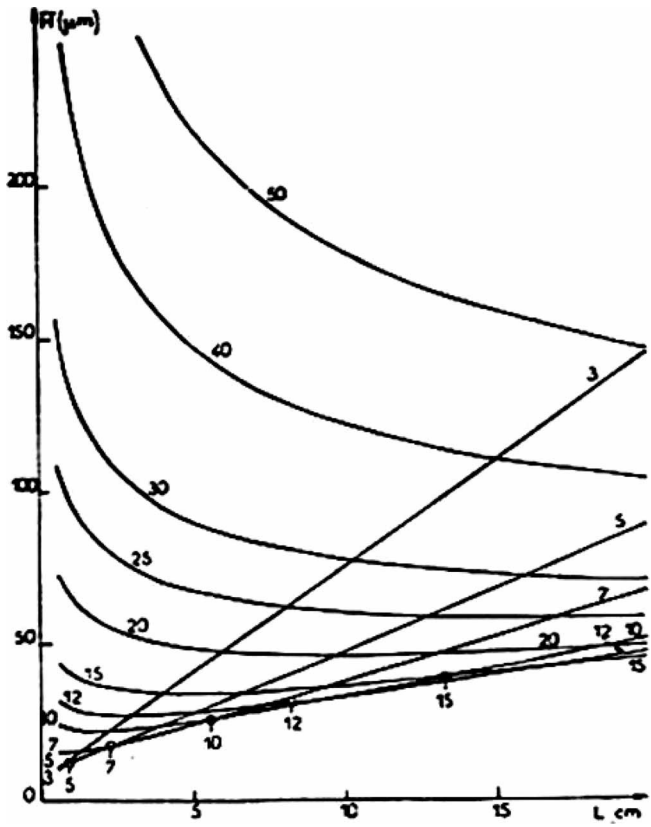

Figure 6. Plot of HETP versus development length for various particle size. The number on each curve is particle diameter. Diffusion coefficient $10^{-5}\,cm^2/s$ (reproduced with permission from Ref. 33, the *Journal of Chromatograpic Science*).

Furthermore the authors demonstrated that the solute diffusion coefficient is a key parameter. These features result in poorer kinetic performance than HPLC (number of theoretical plates, peak capacity). The peak or spot capacity in TLC never overpasses 12–14 in practice.

Fine particle layers provide fast and efficient separations but the requirements are small variance from the spotting technique and efficient slit-scanning densitometers. Manual application of samples can be carried out with micropipettes, capillaries, or syringes. These devices fill up automatically on dipping them into the sample solution and empty themselves on contact with the sorbent surface. This contact application can be reproducible with an instrument such as Nanomat (Camag) but the selection of solvent is critical since it should be as less eluting as possible to prevent band broadening of the spot at the sample deposition. In the 1960s Camag marketed a new sample application method based on the spray-on technique of the sample onto the plate. An atomizer operating from a controlled stream of nitrogen sprays the sample from a syringe, forming narrow homogeneous bands on the adsorbent layer. This application in bands allows applying large volumes, it is less dependent on the solvent, and it improves separation. Automated application allows exact volumes to be spotted, is time-saving, and complies with GLP software documentation. Small volume application is critical to fully exploit the properties of HPTLC plates.

For that purpose Merck made available the concentration zone plates and Camag marketed the Linomat 3 (Figure 7), which dramatically reduced the variance of sample application. Surprisingly, the use of TLC data to rapidly

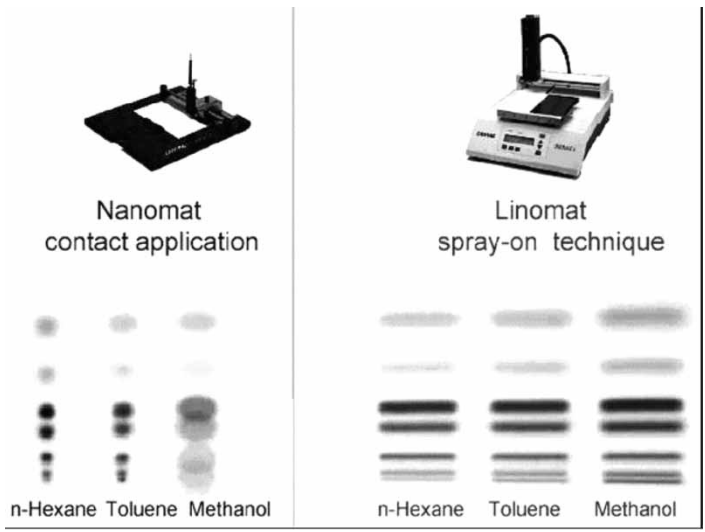

Figure 7. Effect of the sample solvent on the spot size obtained with automated contact and spray-on instruments. Left: spot deposition by contact. Right: band deposition.

select the appropriate HPLC system (28, 29) was not fully exploited at first. Many TLC procedures fulfilled the GLP (good laboratory practice) compliance and were validated in many *Pharmacopeias* (see some examples in reference 30). Optimization methods were reviewed (31). It turned out that one of them, the Prisma developed by Nyiredy in 1985 (32), was really successful and could be applied in HPLC, OPLC, and so forth.

The Multidimensional Capability

TLC exhibits one peculiar advantage over HPLC: it can be multidimensional. In bidimensional TLC, the sample is spotted at the corner of the layer and developed along one edge of the plate, and then the plate is removed from the chamber, dried, rotated at a 90° angle, and redeveloped in the orthogonal dimension with a different mobile phase. This dual process was used for a long time (5). Guiochon et al. (33, 34) derived equations enlightening the full capabilities of bidimensional development. They showed that spot capacity in bidimensional TLC is only matched by bidimensional electrophoresis, and more than 400 spots could theoretically be observed. If the same mobile phase is utilized in the two developments, the spots are lined up in a diagonal. To exploit fully the bidimensional capability of TLC, it is best to use two different chromatographic modes that are the normal phase (NP) and the reversed phase (RP) modes (Figure 8). The first C18 bonded plates were

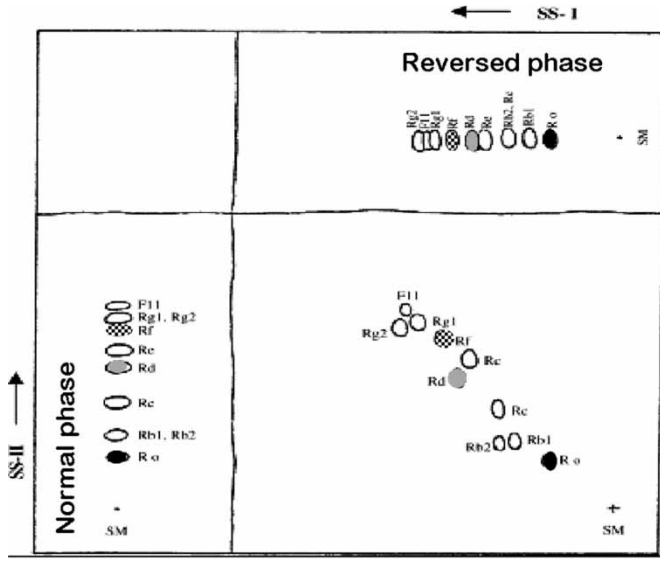

Figure 8. An example of two-dimensional separation (polyaromatic hydrocarbons) with a polar mobile phase (RP) followed by an apolar one (NP).

produced by Whatman in the early 1980s. The same company released a bilayer plate with a C18 strip along a silica gel layer. The success was limited because the separated components tended to focus in the gap existing in between the two layers. Due to band broadening from the second development, bidimensional TLC is not commonly used. It shows great interest either in research labs or to check the stability of compounds in industrial labs.

Overpressured Layer Chromatography and Automated Multiple Developments

When TLC is performed in the simple linear way with plate placed vertically in a chamber, capillary forces alone are unable to produce the theoretical optimum mobile phase velocity. Some attempts (35, 36) were made to produce a quasi-column development by evaporating the mobile phase at the end of an horizontally placed TLC plate. In the mid-1980s two breakthroughs occurred: over pressured layer chromatography (OPLC) was proposed along with the first TLC gradient capabilities with automatic multiple developments (AMD) (Figure 9). Both techniques were automated but they relied on two different approaches: OPLC is a flat version of HPLC, and AMD is optimizing the power of capillary separation. Following these achievements, a new journal, the *Journal of Planar Chromatography*, first appeared in 1988 and its 100th issue appeared in December 2004.

Preceding AMD, programmed multiple development with a single mobile phase over increasing distances was first reported by Perry (37). The spot was focused each time the solvent front passed over the previously adsorbed spot on the layer. In contrast to the conventional multiple development of TLC plates, the AMD development takes place over many chromatographic runs

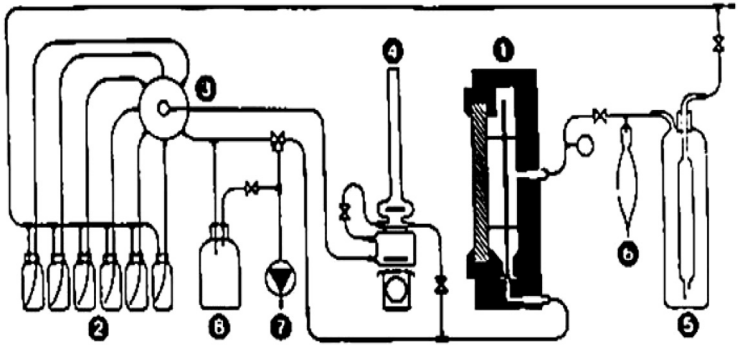

Figure 9. Scheme of the first AMD instrument. (1) enclosed developing chamber; (2) solvent reservoir bottles; (3) switching valve for selecting the solvent composition; (4) gradient mixing chamber; (5) wash bottle for gas phase; (6) reservoir for gas phase; (7) vacuum pump; (8) solvent waste bottle.

of different lengths. The layer is developed over possible 5–35 stages in the same direction for increasing longer distances (3–10 cm) with a stepwise mobile phase gradient of decreasing strength (38). Each new step is running on a fixed distance 1, 2, or 3 mm over the previous one and after a complete vacuum-drying step of the plate. The gradient program may be simple or complex and some nice separations of e.g., pesticides are obtained (Figure 10). Due to the focusing effect, 28 ppt of atrazine in water can be detected on the plate with the technique (38).

There were many attempts to increase the mobile phase velocity (39). The oldest forced flow planar chromatography (FFPC) is reportedly the paper from Hopf (40), who used centrifugal forces. In rotation planar chromatography (RPC), the mobile phase velocity may be varied by adjustment of the plate rotation speed. Some instruments such as Rotachrom or Extrachrom appeared, which were mainly used in the preparative mode. Hostettman (41) described extensively the possibilities of the technique in the isolation of compounds of interest from natural and biological samples.

OPLC was first described by Tyhiak and coworkers (42), who eliminated the vapor phase in TLC by pressurizing the layer. A sealed TLC plate was covered by a flexible inert sheet, which was subjected to overpressure and the mobile phase is pumped through the sorbent layer. A small trough ensured a homogeneous flow. In this mode the system worked like a flat-bed capillary column (Figure 11). Since the time to perform a separation was significantly shorter than in conventional development, diffusion effects were reduced producing small and compact spots. The instrumentation requires a pump to deliver the mobile phase. The commercially available system was a cassette-type apparatus where the prepared TLC plate was

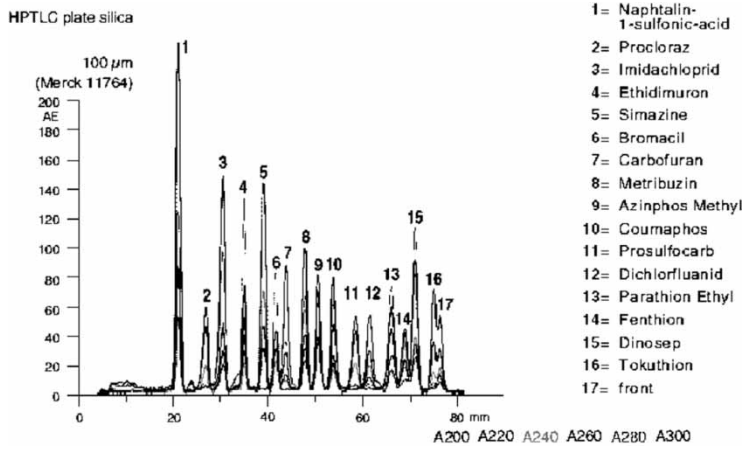

Figure 10. An example of pesticide separation with AMD (from D. Burger presented at HPTLC 2003, Lyon, courtesy of Camag).

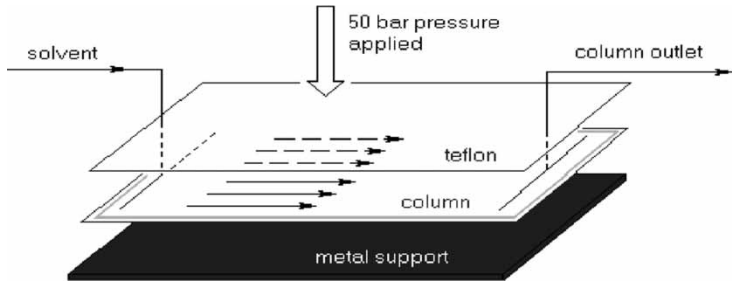

Figure 11. Principle of overpressured layer chromatography (OPLC, by courtesy of Bionisis).

placed in a holder that was inserted through a slot into the instrument in the same way as a CD in a computer.

When the mobile phase outlet of the pressurized chamber was connected to a flow-cell detector, it was possible to detect and collect the eluting solutes. An overpressure of up to 50 bars could be used, which allowed obtaining flow rates matching the optimum linear velocity of the mobile phase (100–125 μL/min) (Figure 12) (43). Actual plate heights in the range 10–30 μm can be obtained for all solutes on the same plate (Figure 13). Comparison of the relevant parameters of OPLC, TLC, and HPTLC has been performed by Nyiredy (44). OPLC can be operated according to many ways: fully off-line, fully on-line with off-line or on-line sample application. A consequence of the constancy of plate height is the fact that position of the sample deposition has no influence on the performance of the system. Many development modes are possible: linear, bidirectional linear, circular, anticircular, and two-dimensional.

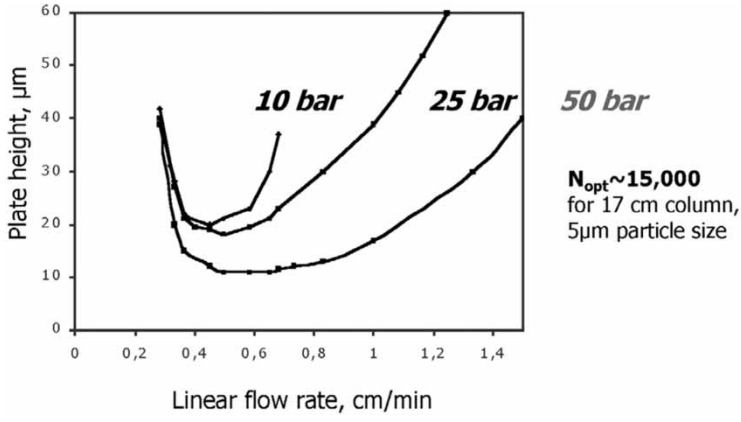

Figure 12. Influence of applied pressure on performances expressed in plate height in OPLC (by courtesy of Bionisis).

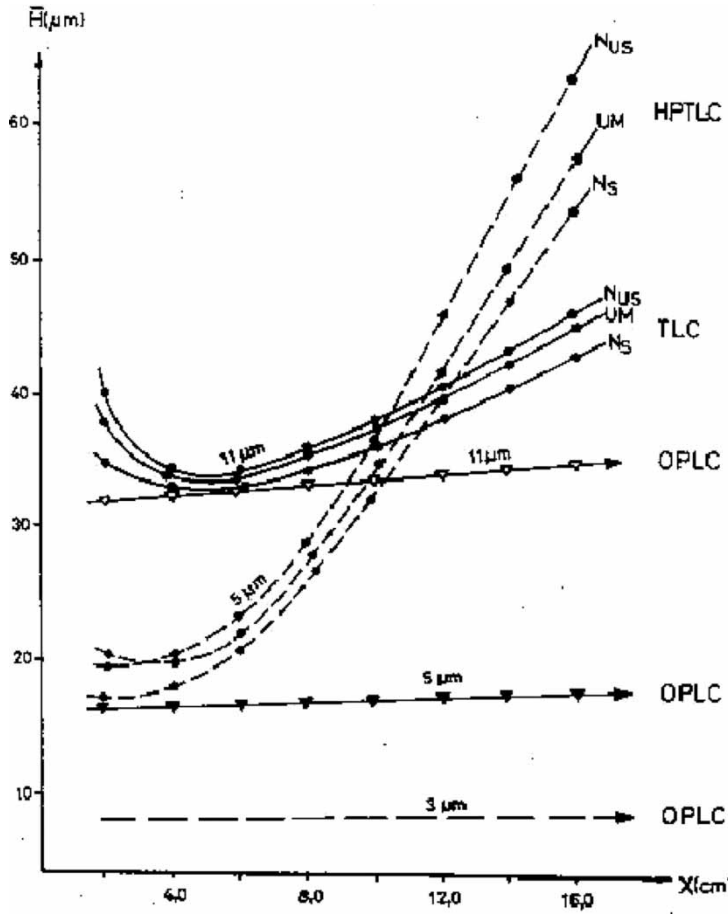

Figure 13. Plot of the average HETP and distance travelled by the mobile phase on different silicagel layers and different chamber systems (reproduced with permission from Elsevier, Ref. 43).

Comparing OPLC and HPLC

An interesting feature that has not yet been fully exploited is the multilayer mode in which several plates are stacked face-up, on top of each other. Excellent reviews on OPLC appeared recently (45–47). In OPLC when the solvent is forced through a dry layer of fine particles, the air displaced by the solvent gives rise to a beta front that is wavy. To overcome this problem, a predevelopment of the layer with a weak solvent is efficient. From the published data (48), OPLC separations take a longer time than similar HPLC separations. However, compared to an HPLC column, the TLC layer exhibits a better packing structure (lower A term of the

Van Deemter equation) but a poorer mass transfer kinetics (higher C term). Also, the TLC layer exhibits smaller values for the total and intraparticle porosity and comparable values for interparticle porosity. Those results suggest that the packing densities for HPLC columns and OPLC layers are similar, but a substantial amount of binder partly fills the pores.

A recent renaming for OPLC is Optimum Performance Laminar Chromatography. To eliminate the edge effects, the flowing eluent wall (FEW) procedure (49) divides the sorbent bed into active and nonactive parts and is used to confine the sample to the active region, away from the edge. In this process, the mobile phase is split upstream of injector so that part of the solvent will go directly to the injector and carry the sample through the active part of the column, while the other fraction of sample-free solvent arrives directly on the column (by-passing the injector) and is directed to the column edges where edge effects occur. In this mode the nonhomogeneous part of the layer can be excluded from the separation process (Figure 14).

THE PRESENT

Today, stationary phases are similar in PC and HPLC and correlations of retention are possible. Silicagel is still by far the most widely used adsorbent

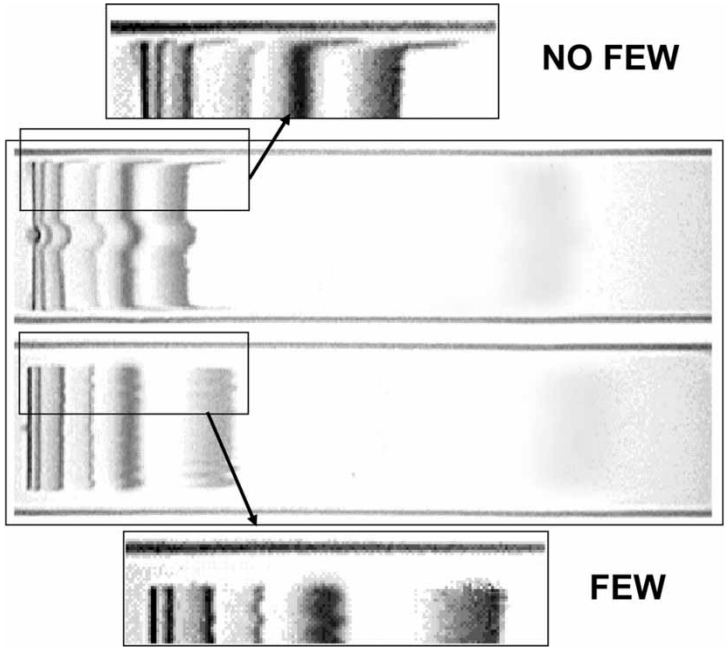

Figure 14. An example of the obtained chromatogram with FEW (by courtesy of Bionisis).

and the normal phase mode with organic mobile phases remains the dominant mode in PC while reversed-phase is the dominant mode in HPLC.

Chiral Separations

When chiral separations are considered, the small number of PC papers is striking. Chiral separations are too often a trial and error procedure and one may think that PC could be a valuable tool for test. Analysis by chiral HPLC gives good separation of enantiomers but the analysis time may be long. The ligand exchange technique with copper (II) complex of N-(2-hydroxydodecyl)-4-hydroxyproline is practically the only impregnating agent utilized by manufacturers (Macherey-Nagel and Merck) to prepare chiral plates when more than 50 different HPLC chiral columns are commercially available. It seems that derivatives of polyacrylamide and cellulose or amylose are not fully exploited in TLC as pilot technique for preparative purpose. Lepri and Del Bubba reviewed the state-of-the-art in chiral TLC (50).

Sad, but Clear, HPLC Domination

A survey of the biannual review on TLC by Sherma in Analytical Chemistry (51) is very informative. Table 2 reports the number of papers published on the PC subject. The decline is obvious if one remembers that in the 1965–1985 period the number of published TLC papers was between 400 and 900 each year. The situation is worse when considering symposia. With the exception of the Planar Chromatography Symposia traditionally held in Hungary, PC communications in major symposia are scarce. However the attendance at the international HPTLC symposium held in Lyon in 2003 (www.hptlc.com) and entirely dedicated to PC was impressive. It should be noted that this meeting was the ongoing of the Interlaken symposia after a 6-year break. Main areas of classical TLC and PC are life sciences and food analysis (Figure 15).

Table 2. Papers published on planar chromatography

Year	Number of articles
1990	470
1994	490
1996	477
1998	325
2000	310
2004	210

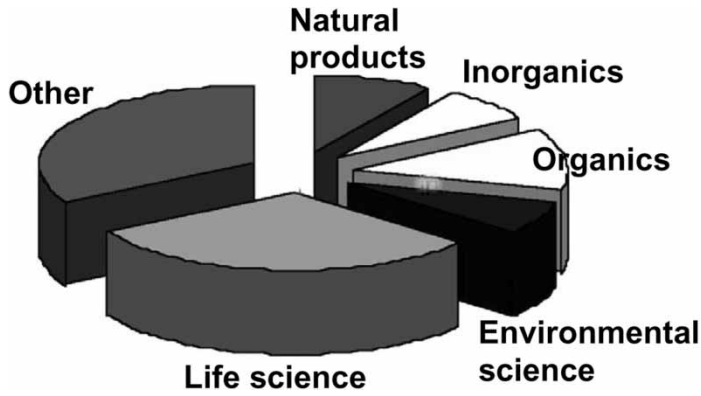

Figure 15. The different application fields of the 210 articles using PC published in 2004.

Opposed to HPLC, PC was marginalized because its benchmarking capabilities and its ability to analyze several samples at the same time providing quick fingerprint profiles were missed. The bottleneck of classical TLC is the time elapsed between chromatography per se and detection of the separated zones. In all cases the plate must be removed from the chamber, dried, and placed in the detector. Conversely, it may be argued that the plate is a storage device that can be used for many detection modes. Off-line and on-line approaches exhibit pros and cons and the discussion is never ending.

A Wide Variety of Detection Modes

Slit-scanning densitometers operating in the UV-VIS absorption mode and fluorescence mode are commonly available for recording TLC separations. Major improvements in this type of instrument seem unlikely. Photodiode array detector allows simultaneous multiwavelength detection (52). Videodensitometric detection is gaining acceptance. The first paper on the video evaluation of TLC chromatograms appeared in 1991 by Prosek (53). He developed his own software and designed the quantitative TLC using a video camera in 1997 (54). The main attraction of image analysis is the fast data acquisition from the whole plate with software that allows comparing chromatographic images (55). It seems that videodensitometry cannot compete with densitometry in terms of sensitivity since only one single wavelength is available. Progress in the field of software and CCD sensors will soon make the use of image libraries possible.

FID detection is still a marginal detection mode. In the 1970s, Okamura and Kadono patented a TLC rod method using 2 mm i.d. quartz rods, coated with 5 μm alumina or silica. These rods could be scanned by a FID detector. The Iatroscan was produced as the first commercially available

TLC-FID (Iatroscan Lab, Tokyo). The system is still in use today. It combines the resolution capacity of TLC with the fast and quantitative response of the FID detector. The technique is suited to solutes such as glycerides or surfactants that do not have native or derivatized chromophores (56, 57).

With Coming Mass Spectrometry PC Detection

Today mass spectrometry (MS) is the best detection technique in gas chromatography (GC-MS) and HPLC. This is not the case in PC. The first report on hyphenation of TLC to MS dates back from 1977 (58). There were some tricks such as the use of a strip of double-faced masking tape to cover the tip of a fast atom bombardment (FAB) probe and press it against the TLC spot of interest, "scrape and elute" is extensively advocated. A review by Wilson (59) describes the attempts to combine TLC with FAB-MS and also liquid secondary ion (LSI) and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) or electrospray ionization (ESI). To perform FAB-MS, it is necessary to use a liquid matrix compatible with the stationary phase such as glycerol or meta-nitro-benzyl alcohol to induce ionization and solute transfer. FAB is not well suited for trace analysis. In recent years there has been an increased interest in the use of MALDI-MS for the direct analysis of TLC plates. In a typical MALDI analysis, the solute of interest is desorbed from a surface in an excess of matrix using a pulsed nitrogen laser at 337 nm. The resulting gas-phase ions of the matrix and the analyte are usually determined by a time-of-flight (TOF) mass analyzer. Application of the matrix onto the plate must be performed in such a way to maintain the chromatographic integrity of the analyte spots.

Parameters influencing sensitivity and lateral analyte spreading are extraction solvent selection, extraction time, and pressure (60, 61). A recently developed electrospray matrix deposition method produces a stable signal, reduces analyte spreading, and hence allows obtaining chromatographic as well as mass spectral data scanning of the TLC plate (62).

After TLC separation, a strip of the plate is cut off and mounted onto a MALDI target using double-sided tape. The MALDI matrix is then electrosprayed onto the surface by applying a high voltage to the spray capillary of the device (63). Particle suspension matrices (1–2 μm graphite particles in ethylene glycol) have been successfully applied (64). Quantitative data requires calibration with internal standard 64). Atmospheric pressure MALDI provides improvements since the chromatographic material does not pollute the source as it is the case with vacuum. To generate structural information, postsource decay (PSD) can be performed directly on the separated spots. PSD can be described as the dissociation by metastable decomposition of intact molecular ions that had gained excess energy during the desorption process.

As was pointed out by Van Berkel et al. (65), TLC-MALDI-MS has some limitations, especially the vacuum chamber of the instrument. Electrospray ionization is extensively used in LC-MS and not fully exploited in TLC. Luftmann described a device that allows for the recovery of compounds from TLC plates and coupling with ESI-MS (66). Van Berkel et al. (65) utilized a combined surface sampling probe electrospray emitter previously described by Wachs and Henion (67), for the direct read out of commercial C18 plates by ESI-MS. Two interfaces developed to connect TLC with ESI-MS on line consisted of (i) two bound optical fibers inserted into the bonded C18 particles at the exit of a small TLC channel and (ii) a small commercial TLC strip with a sharpened tip. Sampling of the plate surface was performed either via a manual spot selection (stepping sampling mode) or a computer-controlled scanning sampling mode. The same group performed the coupling of a preparative rotation planar chromatography system with MS via a simple interface and a self-aspirating heated nebulizer probe of a corona discharge APCI source (68).

Interfaces for coupling TLC with TOF-SIMS were recently described (69). The chromatographic thin layer must be modified to avoid TOF-SIMS background signal activity from the chromatographic material. A possible way is to modify an aluminum-backed plate. A more promising way is the use of a monolithic layer. An on-line TLC-MS interface with ion-trap detection and computer-controlled extraction of solutes from spots permits the control of the amount of extraction solvent and the volume of sample injected (70).

TLC-MS is only used by research groups mainly because the detector price is two orders of magnitude higher than the TLC separation tool. TLC-MS has no scan rate limits, no access order limits, and no analysis time constraints, but PC-MS requires an interface that is not the case in GC-MS or capillary LC-MS. Commercially available TLC-MS systems are still rare. For a discussion, the chapter from Busch is recommended (71). Busch also recently published a review in which he forecasts some possible development in the field (72). A scheme of hyphenating TLC and MS is displayed in Figure 16.

Since OPLC flow rates are within the range of typical flow rates for conventional electrospray, direct coupling of OPLC with QTOF has been demonstrated successfully by Chai et al. (73). For on-line coupling a short piece of capillary PEEK was connected between the outlet of the OPLC and the inlet of the standard electrospray probe. A sensitivity of 5 pmole of glycosphingolipid was readily achieved for PC-ESI-MS and 20 pmole for PC-ESI-MS-MS. Nevertheless the emphasis is on LC-MS and the capabilities of PC are ignored by most analysts working with MS.

And More Exotic Detection Modes

Kovar (74) pioneered the coupling HPTLC-FTIR but applications are still not very numerous (75). Raman spectroscopy is seldom used as a detection

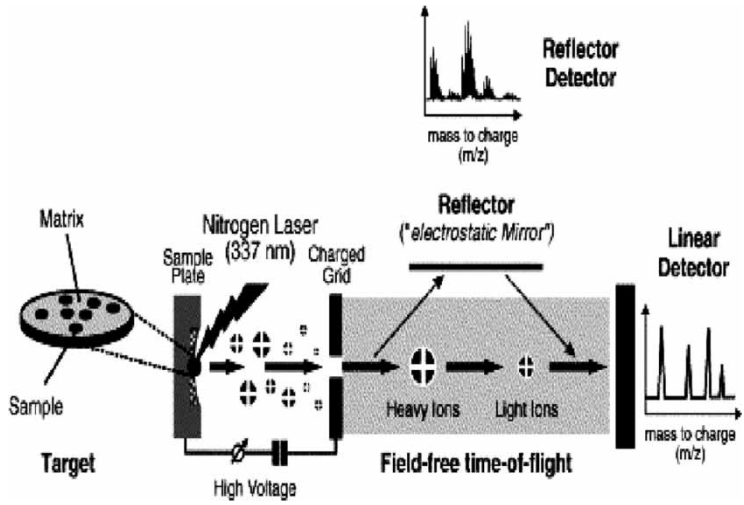

Figure 16. A possible scheme for a TLC −MS interface.

technique. Raman signals are obtained by irradiating a sample with monochromatic radiation and measuring the small portion of scattered radiation that is inelastic. The process is not very efficient. When colloidal Ag is applied onto the adsorbed analyte spots, surface-enhanced resonance spectra (SERS) are recorded with a multichannel micro Raman spectrometer (76) in the near IR range. TLC-SERS can be an alternative to some immunoassays (77).

One advantage of PC and particularly TLC over HPLC is the wide variety of possible detection modes that are difficult or impossible to perform with column chromatography. For example digital autoradiography (78), photoacoustic detection (79), or fluorescence line narrowing that requires low temperatures ($<30^{\circ}$ K) (80) cannot be used in HPLC and gave results in PC.

Poole, in his review (81), pointed out the potentialities of PC in biomonitoring with the wide range of microbiological possible detections. He quoted some 14 relevant references related to isolation of bioactive compounds from medicinal plants, isolation of radical scavengers in foods, screening of combinatorial libraries, etc. A complete chapter on detection of microbiologically active compounds appeared in the retrospective view for the 3rd millenium (82).

THE FUTURE

PC has many unique features that cannot be tackled by any other separation technique. It can analyze a large number of samples. It can preserve the whole chromatogram with the possible use of many detection modes. It can perform sample cleanup and fast separations performed in one single run.

Its bidimensional capability is only surpassed by gel electrophoresis. Planar chromatography is an excellent tool not only for chemists but also for biologists, physicians, and all people working in quality control. OPLC and HPTLC are now making their own headway.

HPTLC will certainly find a niche when other separation methods fail; or when an orthogonal method is required to confirm the result provided by a standard method. Gradients are extensively used in HPLC, the theory has been devised and HPLC optimization softwares are increasingly sophisticated (83). In spite of the early works of Soczewinski's group and the availability of AMD, the theory of gradient in PC remains in its infancy.

Analysts wonder about the future of electro-driven planar chromatography. Electrosmosis is the movement of liquid over a solid under the influence of an applied voltage. TLC with electro-osmotic flow was first described by Pretorius in 1974 (84). One result of applying electric field for solvent migration is that Joule heat is generated that causes evaporation of solvent from the surface of the layer. It was presumed to be the phenomenon that dragged the mobile phase in vertically mounted layers (85).

Electroosmotic flow is generated with polar solvents or aqueous buffers that precludes the use of the NP mode. Interesting results were obtained by Nurok et al. (86, 87) with reversed-phase layers with preadsorbed buffer ions and aqueous–polar solvent mixture as mobile phase. With conventional silica plates and application of a 200 V/cm field, solvent migration rates of 0.039 and 0.21 cm/sec can be attained with ethanol and acetonitrile, respectively. When horizontal chromatographic chambers were used, Nurok et al. obtained 5500 theoretical plates with a 7 cm migration distance. Malinowska (88) investigated the influence of the electric field on the migration of mobile phase into dry silica. The horizontal DS chamber can be closed to perform electrochromatography with 2 kV applied to a 10 cm plate (89). Kreibik et al. (90) designed a vertical chamber for planar dielectrochromatography, a technique that makes use of asymmetric alternating electric fields. Many questions remain unanswered such as the role of the binder or the use of thinner layers.

Shear driven chromatography is a new approach (91). The system consists of two separate longitudinal walls: one longitudinal wall is shorter and is attached to a stationary frame, and the other is longer and is translated (or rotated) past the shorter one (Figure 17). When the longer plate is moved past the shorter plate, the fluid present in front of this channel is automatically dragged in, through, and out of the channel by the viscous effect present in the fluid. The axial velocity in a laminar flow between two flat plates exhibits a linear profile, going from $u_s = 0$ close to the stationary wall to $u = u_w$ near the moving wall. SCD only works if the sidewalls of the channels are perfectly parallel with the displacement direction of the moving wall. The system can be miniaturized (92). The technique is very promising and the cost is very low. Some reversed-phase separations with plate heights as low as 11 μm have been reported.

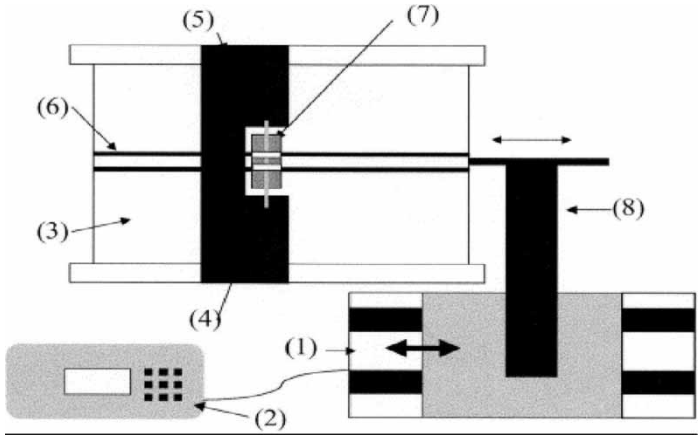

Figure 17. The shear-driven chromatography set-up. (1) axial displacement system; (2) digital controller; (3) moving channel wall; (4) stationary slide holder; (5) guidance rail; (6) printed channel on transparency sheet; (7) SDC column; (8) connection piece (adapted from Ref. 89 with permission).

In HPLC, two major trends are in development: ultrahigh pressure and monolith columns. A thin film of silica-based monolith has been introduced recently by Merck (93). Thin film chromatography is not new. In 1968, Cremer (94) created a vacuum-evaporated indium oxide 1-mm film on a glass plate. The development distance could not exceed 2 cm and a 20-nL spot was a prerequisite. Andreev (95) produced a thin film of fine (2 μm) silica particles and claimed that thousands of plates could be generated. In ultrathin layer chromatography (UTLC), a monolith is grafted onto the glass plate. There is no binder (Figure 18) (96). The

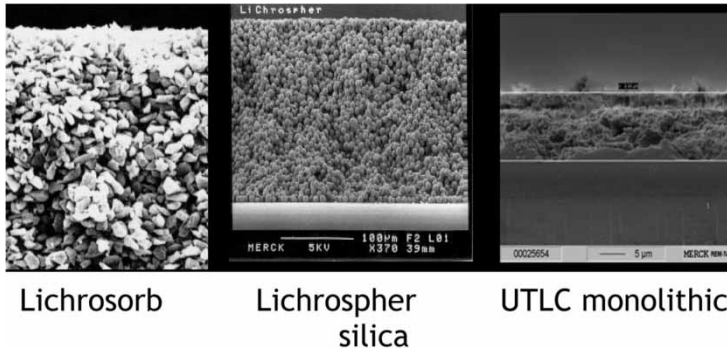

Figure 18. A comparison of TLC, HPTLC, and UTLC silica plates (by courtesy of Merck).

Table 3. Ultrathin layer chromatography

Physicochemical plate properties		
Material	Monolith silica (no particles)	
Format	30 × 60 mm	
Layer thickness	10 μm	
Meso pores	30–40 Å pore diameter	
Macro pores	1–2 μm pore diameter	
Specific surface area	~350 m^2/g	
Specific pore volume	~0.3 mL/g (meso pores)	
Comparing UTLC with TLC and HPTLC		
UTLC	TLC	HPTLC
Sample volume: 5–20 nL	1–5 μL	100–500 nL
Migration distance: 1–3 cm	10–15 cm	3–7 cm
Analysis time: 1–6 min	15–200 min	5–30 min
Solvent used: 1–4 mL	100 mL	20 mL
LOD: 10 pg	1 ng	100 pg

thickness is 10 μm, which requires a very small injection volume (Table 3). Due to the presence of macropores, the capillary solvent rise is short (2 cm) and spots should be very small. Migration distances are in the range of 1 cm (Table 3). The liquid cannot ascend 3 cm due to the macropores. The weakness of UTLC when compared with HPTLC is reduced resolution caused by shorter development distances and a reduced availability of specific surface area.

The demands in the future are for fast, reliable, economical, and analytical methods. The future of planar chromatography will depend on the willing of both researchers and manufacturers. Training in PC in industry or academia is poor and PC is far too underestimated. In 1999 Renger (97) published a benchmark study comparing PC, HPLC, and CE with three possible scenarios. The trend in separation sciences is towards miniaturization and portable instruments. It can be expected that PC will evolve toward that direction.

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