Future Trends

It seems unlikely that video densitometry will ever replace spectrodensitometry as both techniques have unique advantages. On the one hand spectrodensitometry allows the scanning of TLC/HPTLC plates at selectable wavelengths, the acquisition of UV/visible spectra, the determination of peak purity and high accuracy of results. On the other hand, video scanning provides a computer or printed image that can serve as a permanent record of the results obtained which can be documented at any time in a report. Also, for some requirements the accuracy of scanning is sufficient for quantitative evaluation.

With improved software, both densitometric and video scanners are likely to become still more user-friendly. However, more dramatic improvement in the accuracy and reliability of results is more likely to come from the continual improvements taking place in the quality of adsorbents making up the layer. With the introduction of smaller (4 μm) spherical particle sizes, the quality of separation will improve, hence this will be reflected in the scans and quantitative results obtained with both spectrodensitometry and video scanning.

See also: **III/In-Depth Distribution in Quantitative Thin-layer Chromatography.**

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Historical Development

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History

Today the term planar chromatography is commonly used as a synonym for high performance thin-layer chromatography (HPLTC) and conventional thin-layer chromatography (TLC). Originally it referred more generally to a family of techniques including TLC, some types of electrophoresis and paper chromatography, which all have in common a stationary phase in the form of a flat thin layer rather than packed into a column. Modern planar chromatography is a form of liquid chromatography and its history is closely linked to the development of chromatography as an analytical tool.

Early roots go back to Beyrinck who in 1889 separated hydrochloric and sulfuric acid by diffusion through a thin layer of gelatine on a glass plate. With the same technique, Wijsman in 1898 was able to demonstrate the presence of two enzymes in malt diastase. When at the end of the 1930s Tswett's column chromatography became successful, research focused on a faster microchromatographic method, which allowed the exact identification of adsorbed substances. This situation encouraged the transition from a regular column to an open column, a thin layer of adsorbent.

Izmailov and Shraiber are regarded as the inventors of TLC (**Table 1**). In 1938 they described a method in which microscopic slides were coated with 2 mm layers of a slurry made of chalk, talc, magnesium oxide, lime aluminium oxide or other adsorbents and water. On drying, a thin adsorbent layer was formed. The authors investigated belladonna and other plant extracts by placing a drop of the extract on to the layer. This resulted in the so-called ultra chromatogram that was visualized under ultraviolet light. The chromatogram was then developed with several drops of solvent. The most important advantage of the new method in comparison to column chromatography was the short time of analysis and the low consumption of adsorbents, solvents and samples.

Crowe reported in 1941 the use of a microchromatographic method to select suitable solvents for column chromatography. The procedure was

Table 1 Key publications that greatly influenced the development of planar chromatography

Author	*Reference*
Runge	*Der Bildungstrieb der Stoffe*, Oranienburg, 1855
Goppelsroeder	*Kapillaranalyse*, Dresden, 1910
Izmailov and Shraiber	*Farmatsiya* 1938; 3: 1
Crowe	*Industrial and Engineering Chemistry Analytical Edition* 1941; 13: 845
Consden, Gordon and Martin	*Biochemistry Journal* 1944; 38: 226
Kirchner, Miller and Keller	*Analytical Chemistry* 1951; 23: 420
Bate-Smith and Westall	*Biochimica Biophysica Acta* 1950; 4: 427
Stahl	*Pharmazie* 1956; 11: 633
Soczewinski	*Analytical Chemistry* 1969; 41: 179
Snyder	*Journal of Chromatography* 1971; 63: 15

See also publications listed in the Further Reading section.

based on a thin layer of adsorbent poured loosely into a Petri dish.

Meinhard and Hall achieved the first major improvement in layer quality in 1949 with a method called surface chromatography. They used an aqueous slurry of aluminium oxide, Celite and starch coated on microscope slides to produce chromatographic layers free of cracks.

Several years before that, Martin and Synge had taken a different approach while trying to separate amino acids and their derivatives. They developed partition chromatography, in which the stationary phase was coated on to a support such as silica gel and then filled into a column. Solvents such as chloroform were used for elution. In their search for a complementary micromethod, Consden, Gordon and Martin in 1944 used filter paper as an open column, a technique that has its roots in the capillary pictures of Runge (1855) and in the capillary analysis of Schoenbein and Goppelsroeder (1910). Within about 10 years paper chromatography became a universal chromatographic technique.

For the separation of terpenes, Kirchner, Miller and Keller in 1951 improved the surface chromatography technique by incorporating a mixture of zinc silicate and zinc cadmium sulfide as fluorescence indicator into the layer and leaving the Celite out. From various adsorbents to be used on 0.5×5.25 in glass plates called chromatostrips they chose silica gel as the most suitable for their application.

Although employed successfully by many researchers, the thin-layer method was not used widely for several years.

The situation changed with Egon Stahl, who introduced the term thin-layer chromatography in 1956. He realized that the availability of standardized adsorbents of a narrow range of particle size, suitable equipment for preparing thin layers and suitable examples stimulating the use of the method could lead to a breakthrough in acceptance. In 1958 Merck introduced standardized aluminium oxide, kieselguhr and silica gel according to specifications developed by Stahl. Also based on Stahl's ideas, Desaga brought out a basic TLC kit. The wide publicity given to the technique by Merck and Desaga as well as its recognition as a separate chromatographic method by Chemical Abstracts made TLC more and more popular.

In his fundamental book, *Thin Layer Chromatography – A Laboratory Handbook*, published in 1962, Stahl together with several co-authors, made available the results of systematic research on silica gel and other adsorbents, instrumentation and different techniques as well as on the wide applicability of the technique.

The increasing interest in TLC became apparent in a huge number of published papers and several important books, including those by Randerath (1962), Bobbitt (1963) and Kirchner (1967). Due to its much better performance, higher sample capacity and greater flexibility as regards derivatizing reagents, TLC soon replaced paper chromatography in many applications and found entrance to pharmacopoeias as an official method of analysis.

Further developments of planar chromatography were mainly driven by progress in three areas: plate material, instrumentation and theoretical understanding. In 1996 one of the most comprehensive treatises in the field of planar chromatography was published. The *Handbook of Thin Layer Chromatography*, edited by Sherma and Fried, illustrates the current state of the technique.

Plate Material

Experiments with binders were the first step in the improvement of adsorbents that could be made available as pre-coated layers. For reproducible performance, such layers were required to be homogeneous, reliably attached to the support, not affected by solvents and derivatizing agents and universally applicable. Gypsum, which was initially utilized as a binder, failed to yield layers that met the required criteria. Nevertheless, it was still widely used as binder of adsorbents for custom-made TLC plates. In 1965 organic binders based on polyacrylates/methacrylates were introduced which allowed the layer durability and quality to be significantly improved.

Pre-coated plates featuring a wide range of adsorbents, including aluminium oxide, cellulose, polyamide and silica gel as well as a variety of supports such as glass, aluminium or polyester, were introduced to the market. This was a necessary prerequisite for the evolution of planar chromatography into a reliable quantitative technique.

Due to its unsurpassed flexibility and wide applicability, silica gel 60 (average pore size of 60 Å or 6 nm) became the most widely used of all adsorbents.

Paralleling the development in high performance liquid chromatography, an increase in the separation power of TLC plates was expected to result from the utilization of smaller adsorbent particles. In 1975 Merck developed the high performance TLC plate. The main differences of such plates as compared with conventional layers are based on the use of smaller particles with a narrower size distribution. The HPTLC layer is thinner but much more homogeneous and durable than a traditional layer. Separation power and sensitivity are improved and the plates can be used for trace analysis. When introduced to the market these plates became the foundation for instrumental TLC.

A major drawback in the development of layers made with even finer particles to achieve the goal of further improved performance is the restricted capillary flow in such systems. Without suitable forced flow techniques, 3–5 μm seems to be the lower size limit for HPTLC particles.

Partition chromatography on paper was traditionally used to separate hydrophilic substances such as carbohydrates and amino acids. Because these substances are strongly adsorbed on silica gel, reversed-phase techniques had to be employed in order to use TLC. Impregnation known from paper chromatography was used in the 1960s to accomplish this goal. TLC plates were commonly immersed in solutions of alkanes, mineral, silicone or vegetable oils in petroleum ether and then dried. The reversed phase was used with hydrophilic solvents.

When chemically modified silica gel became available, it was attempted to produce true reversed-phase layers for planar chromatography. However, unlike HPLC, which uses a forced solvent flow, TLC has to rely on capillary forces to transport the solvent through the stationary phase. Therefore, reversed-phase materials were developed that retain some of the hydrophilic character of silica gel. Today, a variety of pre-coated reversed-phase plates is available, including RP2, RP8, RP18 and phenyl-modified silica gel. Some of these phases can be used with up to 100% water in the mobile phase. Hydrophilic-modified silica gels originally developed for HPLC also found applications in planar chromatography. They offer additional selectivity and are less affected by humidity and the gas phase in the developing chamber than silica gel.

Theoretical Foundation

Martin and Synge have chiefly influenced the basic understanding of the fundamentals of chromatography. The significance of their work was acknowledged when both researchers were awarded the Nobel prize in chemistry in 1952. One term defined by Martin and Synge in their theory of chromatography in 1941 was R as the relative rate of movement. LeRosen used a similar term for adsorption chromatography. In 1944 Consden, Martin and Synge named it R_F as the ratio of rate of movement of the adsorbate zone and rate of movement of the developing solvent. Bate-Smith and Westhall defined another important term, R_M, in 1950.

These terms, R_F and R_M, are of fundamental importance for TLC and today many publications deal with the significance of these values. Very early in the development of TLC, Brenner, Niederwieser, Pataki and Weber offered an in-depth presentation of the theory relevant to the principles of TLC as a chapter in Stahl's book. One of the recent and most detailed discussions of all theoretical aspects is found in *Fundamentals of Thin Layer Chromatography* by Geiss, published in 1987. Currently, research into further theoretical understanding of planar chromatography is published in the *Journal of Planar Chromatography*.

An important difference of planar chromatography from column chromatography is the presence of the gas phase. Stahl summarized the effects of chamber saturation on chromatographic separation in 1962. Attempts to control the effects of the gas phase have resulted in a number of special chambers, such as twin trough chamber (CAMAG), BN chamber (Desaga) and horizontal developing chamber (CAMAG). The underlying theories of these devices have been reviewed and compared in detail by Geiss.

In 1940 Trappe introduced the eluotropic series as an arrangement of solvents according to increasing elution strength. Stahl incorporated the concept into one of the first practical approaches to a systematic method development. He linked the three main components of the TLC system – activity of the stationary phase, polarity of the mobile phase and polarity of the sample mixture – in a scheme in which the corners of a triangle point to appropriate combinations that promise successful separations. For example, a lipophilic sample mixture requires a nonpolar mobile phase and a highly active stationary phase, whereas a hydrophilic sample can be separated with

a polar mobile phase on a deactivated stationary phase. Soczewinski mathematically described the effects of the solvent composition on the R_M value in 1969.

In 1971 Snyder published a systematic approach to adsorption chromatography. He related the resolution of the chromatographic system R_s to the selectivity α, the layer quality characterized by the number of theoretical plates N, and the position of the sample in the chromatogram. Because of the fact that for a given separation the TLC plate and its performance are usually constant, resolution can chiefly be improved by changes in the mobile phase. The solvent strength influences the position of the sample in the chromatogram and the solvent properties can change the selectivity of the system. Solvent strength was discussed extensively by Rohrschneider in 1973, while Snyder in 1974 published the so-called selectivity triangle describing proton donor, proton acceptor and dipolar properties of solvents commonly used in chromatography. For HPLC Snyder, Glajch and Kirkland developed a detailed model for solvent optimization in the early 1980s. This model was adapted for use with TLC by Geiss. Nyiredy, Erdelmeyer and Sticher proposed a similar but more empirical approach to solvent optimization in planar chromatography, the so-called Prisma model, in 1985. Because of its simplicity, usefulness and wide applicability the model was readily accepted for method development in TLC.

Recently, models for computer-aided optimization that are more theoretically based have been applied to TLC. They include window diagrams (Wang, 1990), simplex optimizations (Sabate and Thomas, 1984), overlapping resolution maps (Glajch *et al*., 1980) and cluster analysis (Windhorst *et al*., 1990).

A useful parameter to characterize the performance of a planar chromatographic system is the separation number, SN, introduced by Kaiser in 1976. In the following years Guichon and Siouffi investigated the relation of SN to height equivalent to one theoretical plate (HETP).

The separation power of planar chromatography is limited due to the capillary flow of the solvent and the resulting limitations in available separation distance. Several attempts have been made to improve performance; One of these is continuous development. The technique, first proposed by Brenner and Niderwieser in 1961, and later by Soczewinski in 1986, is based on the assumption that resolution of planar chromatography can be improved if the solvent is evaporated at the upper edge of the plate, causing continuous development of the chromatogram.

Another approach, multiple development, involves three techniques:

1. Repetitive development with the same solvent in one direction was first theoretically evaluated by Thoma in 1963.
2. Development with one solvent in one direction followed by a second development perpendicular to it with another solvent (two-dimensional TLC) goes back to the work of Consden, Gordon and Martin (1944), who used the technique on paper to separate amino acids. The concept was re-evaluated by Guiochon *et al*. in 1983.
3. Repetitive development with the same solvents in the same direction over increasing distances was introduced by Perry *et al*. in 1975 as a technique called programmed multiple development (PMD). In 1984 Burger modified the technique, in that he developed the plate with solvents of decreasing strength over increasing distances. The term AMD (automated multiple development) distinguishes it from Perry's technique.

The creation of a forced flow through the layer was the third way of improving the performance of planar chromatography. The first experiments to use forced flow in planar chromatography go back to Hopf, who in 1947 invented the Chromatofuge, a device using centrifugal force to accelerate the flow of the mobile phase. Tyihak, Mincsovics and collaborators introduced another concept, overpressured layer chromatography (OPLC), in 1977. OPLC is of special importance in the development of planar chromatography because it can be regarded as a hybrid between planar and column chromatography, combining the advantages of both techniques.

Instrumentation

The first instruments or tools specifically for TLC that appeared on the market were plate-coating devices, developing chambers derived from paper chromatography tanks and sample application tools.

In the early days of planar chromatography, plate-coating devices played an important role. They allowed the preparation of more or less uniform TLC layers of variable thickness. Besides primitive tools such as glass rods for spreading suspensions of adsorbents, there were two kinds of instruments. In one kind (Desaga, Shandon) a dispensing unit was moved over a series of glass plates. In the other kind (CAMAG), glass plates were moved beneath the dispenser. There have been motorized versions of both types of instruments. Even though some are still on the market today, such devices have lost their importance with the arrival of pre-coated plates in the late 1960s.

In the following paragraphs other instruments are discussed according to their designation: sample

application, chromatogram development and chromatogram evaluation. For a review of today's state-of-the-art instrumentation, refer to the chapter on planar chromatography – instrumentation.

Sample Application

Hand-held micropipettes and microsyringes in connection with application guides were the first devices used for sample application. Some of them can still be found in TLC laboratories. However, it was soon recognized that sample application was the bottleneck with respect to staffing levels in planar chromatography. Until the introduction of HPTLC material with its need for miniaturization, all attempts to rationalize sample application were based on the arrangement of dosage devices in parallel and their simultaneous actuation. The Morgan applicator (1962) used a series of capillaries, which were simultaneously filled and discharged by manual action. A number of automated devices (CAMAG, 1973; Shandon, 1974; Desaga, 1976) used a series of syringes or peristaltic pumps, which delivered the sample solutions controlled by an electric motor. The degree of automation of the various steps, syringe filling, positioning and rinsing varied from device to device.

With the advent of HPTLC, automatic sample application had to be performed sequentially by one dispensing device. The CAMAG Automatic TLC Sampler I (ATS1) was the first instrument of this kind. It was succeeded by the ATS II and finally by the redesigned ATS3, a fully automated device for sample application in the form of spots or bands. Desaga developed a similar instrument the AS30 which works in conjunction with a commercial HPLC autosampler.

Chromatogram Development

Vertical development of one or more plates in rectangular tanks, sometimes covering the stationary phase with a counterplate to form a so-called sandwich, is commonly regarded as the classical technique of planar chromatography. Exclusively, capillary forces drive the mobile phase. However, beginning in the early 1960s, attempts have been made to design chambers for horizontal development (Brenner; Niederwieser-Chamber, Desaga). The Vario-KS chamber (CAMAG) was designed to optimize development conditions. The short bed continuous development (SBCD) chamber was introduced by Perry in 1979 (Regis).

When HPTLC plates, which can only utilize short developing distances, became available, Kaiser developed the U-chamber for circular development (CAMAG, 1976; **Figure 1**). There was considerable interest in the device. Even densitometers that could evaluate circular chromatograms appeared on the market. Kaiser also postulated another developing mode, anticircular, which had other advantages and merits to the circular mode. It is possible that this caused some confusion among potential users of both the new techniques and within a few years the devices disappeared off the market.

Figure 1 (See Colour Plate 29) CAMAG U-chamber (1976) developed by Kaiser.

Today interest is focused on chambers that allow automated development with reproducible results. One example is CAMAG's automated developing chamber (ADC). Several, mostly experimental instruments have been constructed for OPLC. Today there is still considerable interest in the technique, especially since a modern instrument is on the market (OPLC-NIT, Engineering Company). Another interesting forced flow technique, high pressure planar chromatography, using the circular developing mode and much higher pressure, was proposed by Kaiser. Unfortunately it was never able to grow beyond the experimental stage.

Modern devices using centrifugal forces to generate a forced flow of the mobile phase through the planar bed are the Rotachrom (Petazon) and Chromatotron (Analtech). Both instruments are primarily used for preparative separations.

Densitometric Evaluation

In the 1960s planar chromatograms were evaluated quantitatively with the help of electrophoresis photometers, some of which had to be modified. The major disadvantage of these instruments was the fact that they used visible light and therefore only coloured substances could be measured. This required some sort of visualization technique to be used.

The first densitometers specifically designed for TLC were those by Zeiss (1965), Shoeffel (1967), CAMAG (1978) and Shimadzu (1979). These devices

were fitted with a monochromator, utilized ultraviolet light and measured in the reflection mode, which has advantages over the transmission mode.

In 1976 the first experimental set-up for evaluation of planar chromatograms by video technology was described by Devenyi. In the following years instruments originally designed for the evaluation of electrophoresis gels were adapted for use in TLC. TLC-specific video systems have been available since 1988 (Uniscan, Analtech).

Modern scanning densitometers as well as state-of-the-art video densitometry are discussed in the section on Thin-layer (planar) chromatography – instrumentation.

See Colour Plate 29.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Instrumentation; Layers; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal; Spray Reagents; Theory of Thin-Layer (Planar) Chromatography.

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Instrumentation

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Introduction

At the end of the 1950s thin-layer chromatography was introduced as a rapid, simple technique for qualitative investigations, and it is still widely used in this capacity. With the introduction of high performance plate material (HPTLC) and the availability of dedicated densitometers, thin-layer chromatography has gained increasing acceptance as a quantitative analytical tool. However, to unlock its full potential as an accurate and reliable technique, HPTLC demands the use of instrumentation for the entire process including sample application, chromatogram development and chromatogram evaluation. Since analytical procedures are only as good as their weakest step, additional operations such as *in situ* pre- or post-chromatographic derivatization, as well as sample preparation, require instrumentation for increased reproducibility.

Although several attempts have been made to automate fully the complete TLC process, it seems questionable whether such automation would increase the overall performance of the method. It has proved very difficult to design a device, at a reasonable cost, that is essentially an automated online system, but at the same time retains the extreme flexibility of the traditional offline design of TLC. One of the many distinct advantages of TLC – the fact that each step can be separated in time and location from all other steps – is very likely to be lost in a fully automated design. Automation of each individual step, perhaps linked by suitable software, appears to be the better choice.

Sample Application

As the first step of planar chromatography, sample application largely determines the overall quality of the separation. All analytes and standards are chromatographed and compared with each other on the same plate, generally by means of migration distance or R_F value. Therefore, exact positioning of the sample during application is crucial for both qualitative and quantitative TLC. Quantitative work also requires exact reproducibility of applied volumes. Furthermore, in order to utilize fully the separation power of the layer, it is important to restrict the dimension of the sample origin in the direction of chromatography. The advantage of instrumentation is mainly derived from a much higher reproducibility of all these parameters.

Generally, two principal sample application techniques are distinguished: application of spots and application of bands. The selection of a sample