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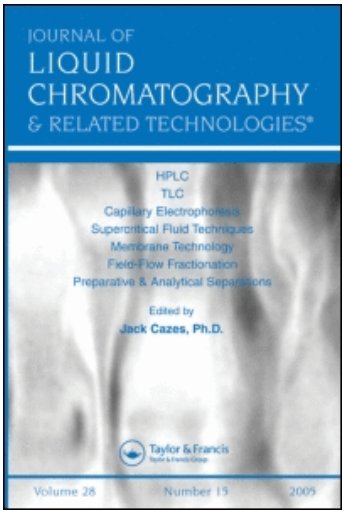

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Recent Developments in Thin-Layer Chromatography - II

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RECENT DEVELOPMENTS IN THIN-LAYER CHROMATOGRAPHY - II

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ABSTRACT

This review updates the one we did 4 years ago. The emphasis is on technique rather than application. Recent advances in adsorbents, plate shape, developing chambers, gradient elution and quantification methods are reviewed and commented on. Theoretical studies of the basic aspects of TLC processes are also reviewed.

INTRODUCTION

In the 4 years since we reviewed developments in thin layer chromatography (TLC) (1), much has appeared in the literature. This up-dated discussion will concentrate on techniques and recent advances which aid the analytical chemist, rather than applications which can be found in published work of the last few years (2).

Although it is 30 years old, TLC is a modern technique which has undergone much change. Its success has been due to its low cost, ease of operation, speed, and nondestructive nature, when spray reagents are not used. This review will introduce the reader to the most interesting recent developments in this fascinating separation technique.

DISCUSSION

ADSORBENTS: Silica gel is still the most widely used adsorbent. However, the development of reverse phase TLC has grown since its value has been proven in high performance liquid chromatography (HPLC). A recent review of the use of reverse phase in TLC (3), and a study of layers with lipophilic modifications (4) have been published. Plates coated with more than one adsorbent, or in which gradient phases are utilized, have also been introduced. For example, plates coated with silanized silica gel and silica gel side-by-side on the same plate, have been used to separate oxidation products of cholesterol (5). Whatman Co. (6) introduced a plate in which a narrow reverse-phase C_{18} strip is coated side-by-side on a silica gel plate. Stahl et al (7) recently introduced a pH gradient layer. The advantages of two-phase and gradient layers are many, but the main value is for the separation of otherwise unseperable mixtures. Two phase plate may also be used to clean up a sample on one phase and for separation on the other, and for the separation of complex mixtures of varying polarity.

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Two phase TLC plates are formed by spreading two phases side-by-side, by developing a precoated plate in a silylating agent (8), or by grafting two precoated TLC plates together as described by Pandey et al (9). Grafting TLC is a two plate system, (with layers which can be different or the same) in which the plates are clamped together with the edges of their adsorbent layers in contact so that a band on one layer is transferred to the other. The advantage of such a method is that bands on one plate can be transferred to another plate for further TLC analysis without scraping, eluting and reloading. Care should be taken that the point at which the two plates meet is close, or the sample will not be quantitatively transferred. Preparation of the plates should be of the highest order.

Another advance in plate preparation which is gaining momentum is sintered TLC, which was introduced in 1973 by Okumura et al (10). The plate is prepared

by mixing silica gel with glass powder. The slurry is then spread on a soda-lime glass plate and the prepared plate placed in an oven at high temperature to fix the adsorbent layer to the plate. The resultant TLC plate is very hard and can be used repeatedly after cleanup with a chromic acid-sulphuric acid solution. Okumura wrote an excellent review (11) discussing different applications and plate preparations.

Ion exchange layers of strong cation and anion exchange resins which are spread on poly(ethylene terphthalate) sheets are commercially available (Chromatronix, Palo Alto, CA). They are widely accepted and have been used to separate amino acids (12), nucleic acid bases (13) peptides and other ionic mixtures (14) in food and body fluids.

An advance which is widely used is high performance TLC. In this technique the particle size (3 to 8 μm) is smaller than that usually used in conventional TLC, and the size distribution is kept to a minimum, which lead to higher efficiency and less diffusion. As a result, the spots are more compact, and separations are achieved using shorter development distances (15).

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PLATE DEVELOPMENT: Many significant advances in plate development have been introduced in the last few years. They include radial, anticircular, compressed, gradient, and continuous (both at room temperature, and at -77°C using a cryogenic apparatus).

In radial or circular development, which has been used for years in paper chromatography, the mixture to be separated is spotted at the center of a sheet and the solvent flows from a pipette on to the plate and separates the components into concentric circles. Recently, an accurate and relatively simple apparatus, the U-chamber (15), was introduced for use with high performance TLC (HPTLC). In this method the solvent is delivered to the plate at a predetermined flow rate (Figure 1). The sample is either spotted on a 5 X 5 cm plate or injected into the stream of the solvent. If the sample mixture is spotted at the center, or injected into the stream, the

Figure 1: The U-chamber for circular development.

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components separate as circles. However, if the samples are spotted around the center, the sample components separate in arcs (Figure 2). The most accurate system in terms of experimental conditions is the U-chamber by Camag, which uses a micro-syringe attached to a pump to feed the solvent at a constant rate. Development time is about 4 minutes, the amount of solvent needed is less the 1 ml, and the experimental conditions are reproducible.

Two other units are available for circular development; the SelectaSol (Figure 3) by Schleicher and Schuell and the High Performance Radial Chromatography Chamber by Fotodyne Inc. Whereas the U-chamber uses a pump to deliver the solvent at a predetermined flow rate, the Fotodyne unit works by gravity, and the SelectaSol by capillary action. Both units are fast

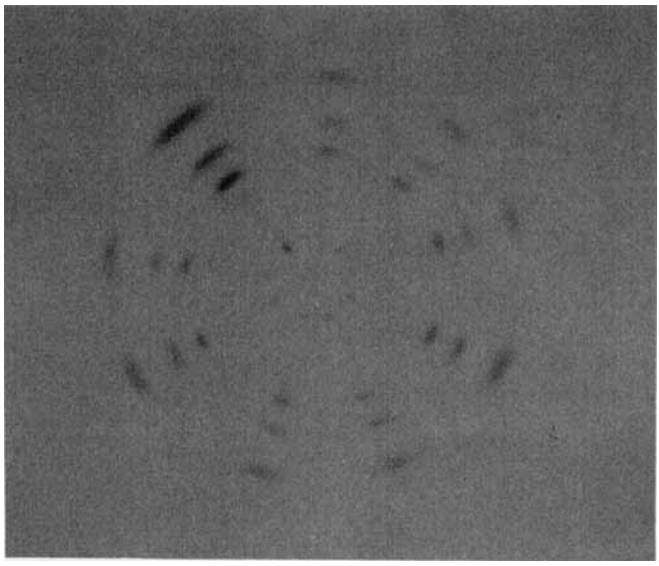

Figure 2: Circular development of a dye mixture using the Fotadyne unit.

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which aids in the selection of a solvent system. With the SelectaSol unit the analyst can simultaneously run a sample in 16 different solvent systems on a 20 X 20 cm plate.

Horizontal circular centripetal TLC was introduced by van Dyk in 1970 (16). In this method the sample is applied at the circumference of a circle, and elution proceeds toward the center. The advantage of this is that the concentration per unit area is increased with decreasing diameter. Also, the separated components can be withdrawn from the center. Later, the apparatus was simplified by Kyne & Vetters (17). Their apparatus consisted of a turntable on which the TLC plate was positioned while the solvent was introduced from a stationary syringe to a felt ribbon surrounding the plate. The system must be protected from drafts, or the circles produced will be irregular. The main problem encountered in centripetal TLC is the transfer of the eluting

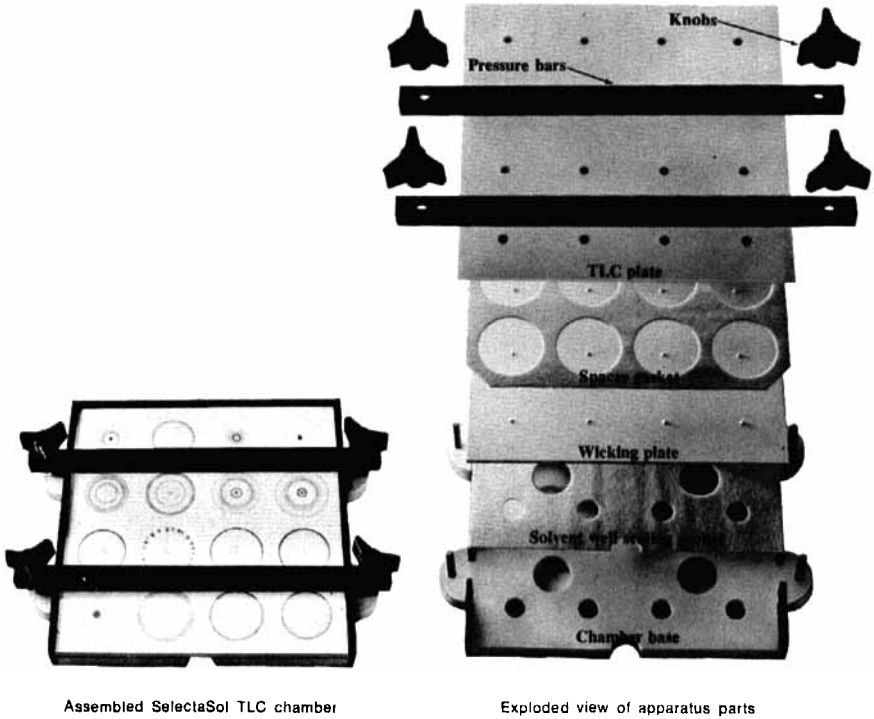

Figure 3: The SelectaSol chamber assembly.

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solvent to the plate. In 1978 a modified U-chamber was introduced by Kaiser for anticircular high performance TLC (18). The solvent is fed into the plate by capillary action from a narrow round channel. A simpler, and far cheaper anticircular TLC developing device was reported by Kariko and Tomasz (19). The device consists of a petri dish into which the solvent is placed. Contact between the round plate and the solvent is accomplished through a cylindrical filter paper strip with feathered edges. This device does not give as reproducible or consistent results as the anticircular U-chamber. Issaq (20) combined the advantages of the modified U-chamber and petri dish unit to construct a simple, inexpensive and reliable anticircular developing apparatus. Anticircular TLC has the advantage over circular TLC in

that a large number of samples can be spotted. Also, the resulting spots are more concentrated, i.e. molecules/unit area, because the available area decreases quadratically with the solvent front. The higher the R_f, the more elongated the spots, which affects the resolution of the mixture. However, the resolution at R_f values above 0.5 was improved by developing the plate, after drying, a second time in the same solvent system (20). This took only a few minutes (Figure 4). Densitometric studies showed not only that better resolution of the spots had been obtained, but more uniform and sharper peaks (20), which are the result of a greater concentration of the spots i.e. molecules/unit area.

Better resolution of sample mixtures was obtained when the sample solution was applied to the plate as a streak rather than a spot. When the sample was applied as a fine band, the resolution of anticircular and triangular TLC was comparable with that of circular development (21).

Soczewinski and Wawrzynowicz (22) developed a sandwich tank in which the solvent is delivered to the TLC plate from a small reservoir by a capillary siphon using a device which distributes the solvent at right angles to the direction of development. The advantages of such a system are that only one tenth as much solvent is used compared with saturated tanks, and the sample is also preconcentrated on the plate (23).

A new system using a pressurized ultra-micro chamber has been introduced (24,25). The adsorbent layer is covered by a membrane under external pressure. The solvent is introduced by means of a pump. The advantages of such a system are shorter development time, less solvent use and performance equal to that of HPLC, since there is no vapor pressure as in standard TLC. It was concluded by Kalaz (26) that more compact spots are obtained in the absence of the vapor phase.

Camag has introduced a linear-sandwich type HPTLC chamber (Figure 5) which uses 10 X 10 cm plates. Samples are spotted on opposite side of the plates which is then developed in the special chamber. The amount of liquid phase

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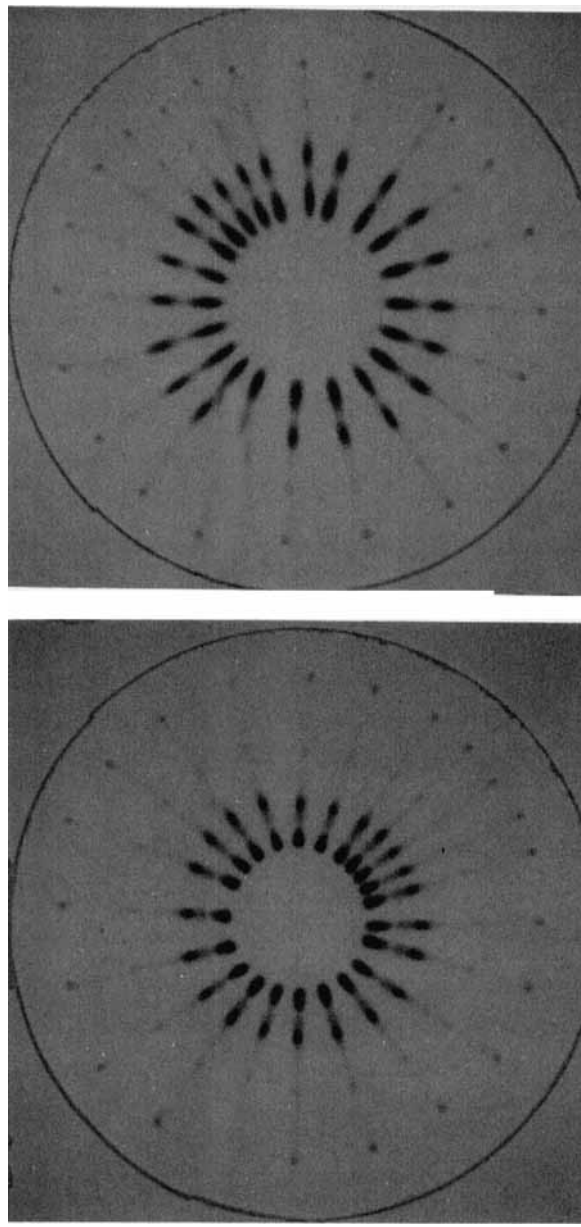

Figure 4: Anticurcular development of a dye mixture, using an apparatus which was developed in the author's laboratory. (a) First development (b) second development in the same solvent system.

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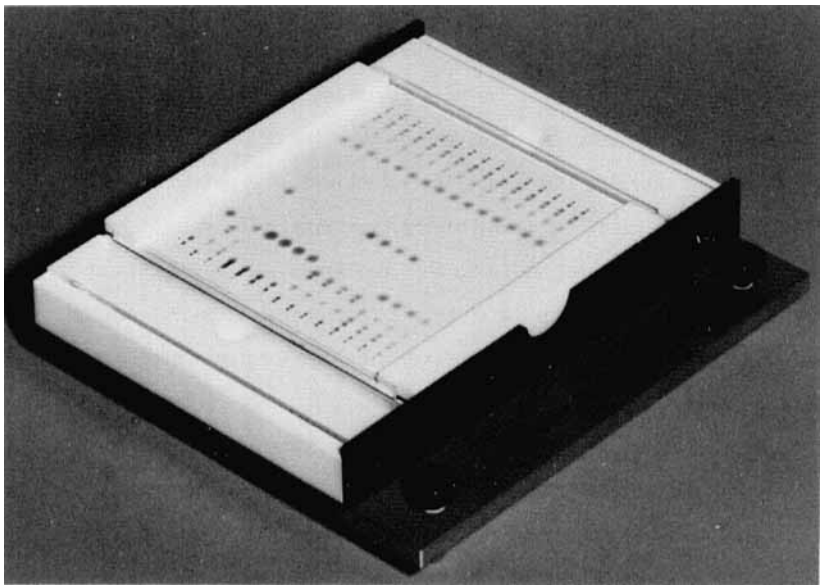

Figure 5: HPTLC linear development unit.

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needed is approximately 1.5 ml. Another model is available for developing 10 X 20 cm plates. The advantages of this chamber over linear TLC in a tank are the smaller volume of solvent, and the ability to develop twice as many samples per plate, since samples are spotted on opposite sides of the plate. This results in a 50% savings in plate use. Again, one has to assume that a short development meets the needs of that particular analysis. Another advantage (27) of this system is in the two-dimensional development of four samples simultaneously, which cannot be done easily using any other TLC mode. The samples are spotted at the four corners of a 10 X 10 cm plate and developed. After which the plate is taken out, dried, rotated 90° and developed again in a different solvent system. The result is the rapid two dimensional development of four samples on the same plate. In classical TLC this would require four separate developments - two in each solvent system.

An apparatus for continuous development (CD) TLC at room temperature (22,28) and at -77°C (29) has been reported. The theory and advantages of CD have been discussed by Perry (28). The main advantage is that it can separate closely related compounds using solvent of very low polarity. The lower the polarity of the solvent, the larger its selectivity. A point of caution may be appropriate here. CD using binary, tertiary, etc. solvent mixtures of varying polarities may lead to solvent demixing. Two approaches which have been reported (30) to minimize this effect are, (a) conditioning of the plate in saturated tanks, and (b) construction of flat tanks of the sandwich type which have a minimum volume.

Continuous development at -77°C enables the separation of conformational isomers which would equilibrate at room temperature. Also more compact spots are obtained (29) which results in improved resolution.

One of the strengths of HPLC is that solvent gradient elution can be used to separate compounds of different polarities. In HPLC, it is easier to achieve such an elution mechanically than in TLC because (a) the solvent is continuously discharged from the column; (b) no vapor equilibrium is required as in TLC; and (c) the size and design of the TLC developing tank is a limiting factor. The last point merits discussion. To achieve reproducible R_f values in TLC, vapor saturation and equilibration in the developing tank must be achieved before plate development. The classical commercial tanks (10 X 25 X 25 cm) are too large to allow vapor equilibrium to be reached in a short time, and they require approximately 100 ml of solvent. Also, if gradient elution is used with the classical tank the design of the tank does not allow easy discharge of the solvent.

Recently, a few attempts have been made to use gradient elution in TLC. Blome (31) used gradient elution with the U-Chamber, which has a small volume and requires only 1 ml of solvent. Soczowinski and Czapinska (32) used step-wise gradient development in conjunction with sandwich tanks. A capillary siphon delivered the solvent at right angles to the direction of development

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which permitted the composition of the solvent to be changed in a simple manner by substitution of containers. Gradient elution on reverse phase plates has been reported (33). Two pumps and a solvent programmer were used to generate the required mobile phase gradient. Three layers of 100 mesh steel powder were used to disperse the solvent evenly in the developing trough, wetting the adsorbent, and allowing plate development. Excess solvent flowed from the trough into a waste solvent container. A four-component mixture was separated using the above system, which seems to be the best TLC-gradient elution system reported to date.

PLATE SHAPE: In TLC, separations are generally obtained by linear development on rectangular plates. Recently, the circular mode of development (including anticircular) is gaining popularity (U-chamber, SelectaSol, pressurized TLC and other variations). The advantages and disadvantages of linear, circular and anticircular TLC have been discussd by Kaiser (18), who compared separation number, sensitivity, analytical separation power, R_f data, consumption of plate material and consumption of mobile phase per sample. These comparisons showed the anticircular mode to be superior to the other two in terms of relative sensitivity, the number of samples per plate, speed of analysis, and amount of mobile phase required per sample. The linear mode was ranked second, and the circular mode last. However, the circular mode did give the best separation number. The separation number can be improved in the anticircular mode by developing the plate twice in the same solvent system. The initial cost of each was not compared, but the linear mode is the cheapest since it does not require the special developing apparatus required by the circular and anticircular modes. In the long run this initial cost will be offset if a sufficient number of analyses are needed. Another important aspect not mentioned is that in circular and anticircular TLC, advantage cannot be taken of two dimensional development. The use of two different mobile phases, one polar and the other nonpolar, may reveal spots not resolved

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by a single development. Also, inefficient and incomplete separation of a multi-component mixture may result if a 3-4 cm run is made on a 10 X 10 cm plate. In classical TLC using a 10 X 10 cm plate and two dimensional development, it is theoretically possible to separate a mixture of at least 50 components which is not possible using circular, or anticircular, development (34). Nor is the analyst limited by the size of the plate which can be used whereas anticircular and circular methods use only 5 X 5 cm or 10 X 10 cm plates.

Recently, Issaq (34) used a triangular plate (Figure 6) which combined the advantages of both the anticircular and linear modes. In triangular TLC the samples are spotted at the base of the plate, which is then developed in a regular tank, (rectangular or cylindrical). The sample, after development, is more concentrated (molecules/unit area) than in any of the other three modes. In triangular TLC the plate is developed twice in the same solvent system which results in compact spots, especially at high R_f values. The advantages of triangular TLC are a 50% savings in plate use compared with conventional TLC, and restricted diffusion of the spots after development. Since the sample is spotted at the base of the plate, the movement of the solvent front is from a wide to a narrow area. As a result the spots are compact and concentrated, (molecules/unit area) which means increased sensitivity (lower detection limits). Preparative separations on triangular plates are preferred over square or circular plates because the sample is streaked at the base of the triangle. After development, the streaks are shorter and less solvent is used to elute the samples from the plate, and no special developing apparatus is required. Plates of any size can be used from 5 X 10 to 20 X 20 cm, without the need for special equipment or attachments. Also, two dimensional development is possible with an equilateral triangular plate.

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MODIFICATION OF ADSORBENT, SOLVENT AND SAMPLE: In previous sections modifications of developing chambers and plates were discussed. Another

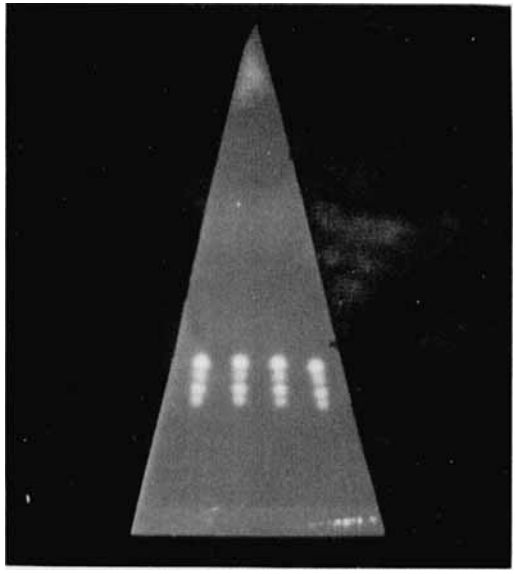

Figure 6: Triangular TLC of aflatoxins B_1, B_2, G_1 and G_2 on silica gel plates developed in chloroform:acetone:ammonia (90:10:0.25).

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aspect of TLC involves modifications of the adsorbent (derivatization, silylation, and impregnation), the solvent (addition of silylating agents, use of micellar solutions and soap chromatography) and the sample (derivatization of the sample). These changes give the analyst a further opportunity to effect the separation of either closely related compounds, or a complex mixture which would be difficult to separate by conventional methods. These modification are discussed in detail in a recent review (35).

QUANTIFICATION IN TLC: Few advances in quantitative TLC have been reported since Camag introduced the HPTLC Scanner for photodensitometric measurements of linear, circular and anticircular plates. Such a scanner has been a great help with these popular techniques.

Another advance which has gained acceptance is the combination of TLC/FID detection and quantification. In this procedure the sample is spotted on a rod with a sintered silica gel or alumina layer (10). After development, the rod is passed through a flame ionization detector (36). The system, Iatroscan TH-10, is manufactured by Iatroscan labs, Inc. Tokyo, Japan. This TLC/FID combination seems to work well and give reasonable results. Since the chromarod is of the sintered type, it can be reused. Background values, which can affect the quantitative measurements, can be controlled by prewashing the chromarod prior to use.

Modified radioscanners can be used to measure the activity of a 1 X 20 cm channel on the plates. The new scanners are connected to a strip chart recorder or a video screen, which gives the analyst a quick evaluation of the chromatogram. Two new models are available from Berthold, Switzerland, and from Bioscan, Washington, D.C. Both systems are sensitive, and efficient, and require no sample preparation such as scraping or cutting. Measurements are made *in situ* on the plate. They both offer good resolution and sensitivity and are quick and easy to operate.

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Another densitometer which uses a video screen is the Telechrom videodensitometer, (Chinion - Budapest, Hungary) developed by Devenye (37). It will be produced in this country in the near future. The unit has a vidicon - tube as the detector, and scans the spots in three dimensions. The X and Y axes correspond to the shape, and the Z axis to the density, of the spot. The size of the spot may vary from 2 X 2 cm to 2.3 X 3.6 cm. Small spots are magnified optically by the instrument.

Microprocessors are used in TLC, in conjunction with fluorimeters and densitometers, for quantification and for data storage and handling. Foss *et al* (38) described an elegant system for the acquisition and reduction of TLC data. The system consisted of a microcomputer, a cassette recorder for storage of data and program, a TV screen for visual monitoring of the chromatogram and an X-Y recorder which serves as a printer/plotter. The microcomputer was

interfaced with a Schoeffel SD 3000 Spectrodensitometer. Either absorbance or fluorescence data may be gathered. Plates may be scanned either in parallel or perpendicularly to the direction of development for best results. The system is versatile and gives the analyst a quick evaluation of his chromatograms. Pollak (39) in a comprehensive review of the use of microprocessors in quantitative TLC discussed data acquisition, reduction and retrieval. He also covered the use of different spectrophotometers (dual and single wavelength), and scanning modes including densitometry (reflectometry and transmission), and fluorimetry. The effect of coefficients of absorption and scatter on quantification were also discussed.

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SPOTTERS: Spotting is still being done with a micro syringe, micropipette or an automatic spotter. The advantage of an automatic spotter is reproducibility of both the amount spotted and the size of the spot, which are both important when quantitative data are needed. A disadvantage is that the unit has to be cleaned after spotting a sample, unless disposable micropipettes are used. The newest sample applicators for both classical and high performance TLC are the Linomatt III, and the nanoapplicator, both by Camag.

The Linomat III (Figure 7) uses an interchangeable micro syringe. The samples are applied to the plate as narrow bands of varying lengths, up to 20 cm. With the nanoapplicator the samples are applied as spots ranging in volume from 10-230 nl. The sample size to be spotted is adjusted with a micrometer to ensure both reproducibility and accuracy.

The application of a large sample, 1-3 ml, to a preparative plate, 0.5 mm - 1 mm thick, is not an easy task. Application as a spot leads to a series of microcircular chromatograms, while application with an automatic applicator as a streak produces two opposite frontal zones with a central zone containing the most strongly retained component (40). Soczewinski and Maciejewicz (41) solved the problem by using a sandwich tank with a glass distributor. The sample is applied to the edge of the plate with the glass distributor which forms a

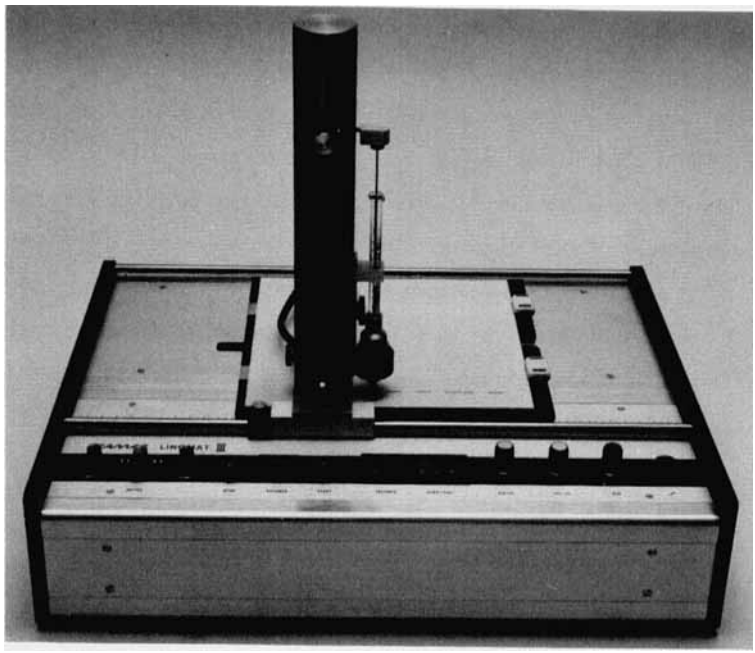

Figure 7: The Linomat III nanoapplicator.

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horizontal flat pipette in contact with the edge of the adsorbent. Partial separation takes place during the application which is then completely separated after plate development in an appropriate solvent.

THEORETICAL CONSIDERATIONS: Recently, there has been much progress in our understanding of the theory of separation, in both partition and adsorption chromatography, and the effects of solvent-solvent and solvent-solute interaction. A few selected works are mentioned in this field. It is recommended

that the reader refer to these works for a more detailed discussion. Solute-solvent interactions on the surface of silica gel and the effect of using polar and non-polar solvents on the solute, as well as their behavior on the silica gel layer and competition for the available sites have been studied (42,43). It was found that a solvent bilayer formed on the surface of silica gel when using hydrogen bonding polar solvents. It was concluded that when a low concentration of a polar modifier was employed, the solutes interacted with a primary layer of polar solvents without displacing the solvent. However, at higher concentrations, the solutes interacted with the primary layer, displacing solvents in the second layer, but did not react with the silica gel surface itself. For solutes with a polarity comparable with that of the modifying solvent, competition for the primary layer can take place, and the solute interacts directly with the silica gel surface (44).

Perry (28) studied the relation between solvent strength, selectivity and continuous development. He concluded that selectivity (the center-to-center separation ability) increased exponentially with decrease in solvent strength. Also, at the high selectivities which become available with decreased solvent strengths, the number of theoretical plates required for resolution became negligible. Spots were then resolved after very short migrations (28).

Soczewinski (44) studied the relation between eluent composition and retention. Equations were presented for the optimization of TLC and HPLC systems. It was concluded that, depending on the system type, the Rm (log K') values are often a linear function of the modifiers concentrations. Martire and Boehn (45) presented a molecular theory of liquid adsorption chromatography. The theory addressed the molecular mechanism, based on lattice models, of retention and selectivity in both normal phase and reverse phase liquid adsorption chromatography. Solvent-solvent and solvent-solute interactions were discussed and practical application of the theory were presented. Chen and Horvath (46) evaluated the substituent contributions to chromatographic retention for quantitative structure-retention relationship. They present

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data obtained with different C_{18} silica gel stationary phases at various temperatures which suggest that quantitative structure-retention relationship can be transformed from one reverse phase to another, as long as the eluent composition is the same. Guiochon et al (47-51) wrote a series of articles dealing with TLC. In their articles they discussed, (a) particle size and its effect on spot shape and size, band broadening and plate height, (b) the effect of the flow velocity of the mobile phase in which it was shown that adsorption of solvent vapor from the gas phase on the dry layer can affect the solvent front velocity, (c) optimization of experimental conditions such as particle size, flow rate, analysis time, (d) effect of temperature, and (e) the flow rate in reverse phase TLC. Guiochon and his co-workers present the TLC chromatographer with clear answers and an easy-to-understand discussion.

Finally, although Snyder's book (52) on adsorption chromatography was published in 1968 it is still a useful reference work for chromatographers using TLC and HPLC. Another good work is "Introduction to Modern Liquid Chromatography" (53) by Snyder and Kirkland, especially chapters 6, 8 and 9.

The optimum composition of a binary solvent mixture in TLC is normally determined by trial. A theoretical estimation of the optimum separation may be obtained from the relationship between Rm values of substances one and two and the composition of the mobile phase (54). The most favorable Rm value can then be measured using an equation which relates the above parameters.

CONCLUSION

This review clearly demonstrates that, in recent years, research into the basic aspects of TLC has been on the rise. These include (a) modifications of the adsorbent (reverse phase C_2, C_8, C_{18}), the sample (derivatization) and the solvent (micellar and soap chromatography); (b) scaling down the size of the adsorbent particles and controlling their size (HPTLC); (c) decreasing the size of the plate (triangular and HPTLC) and changing its shape (circular and triangular); (d) introduction of controlled development modes (U-Chamber for circular and anticircular, continuous development at room temperature and

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in the cold, pressurized ultramicro chamber, and a new sandwich tank); (e) the the use of more than one adsorbent type side-by-side on the same plate; and (f) the use of a solvent programmer for gradient elution.

Theoretical studies of the basic aspects of TLC have been published which improve our understanding of the underlying TLC processes.

The challenge to TLC from HPLC has been met with new and useful advances, which makes TLC as modern a technique, and as helpful and valuable an analytical tool, as ever.

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