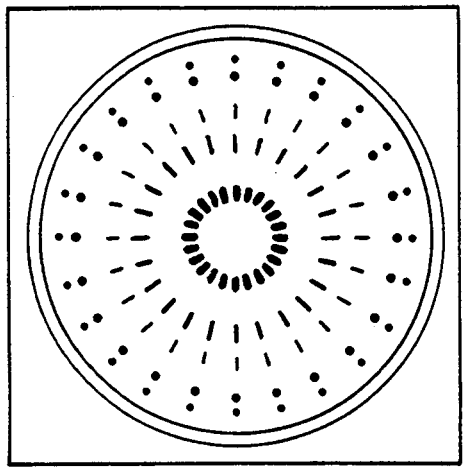

Figure 18 Anticircular chromatogram. (Reproduced with permission from Fenimore DC and Davis CM (1981) High performance thin-layer chromatography. *Analytical Chemistry* 53: 252A.)

with sulfuric acid containing naphthoresorcinol by spraying or dipping with this reagent and heating at 100°C for 5 min. The spots near the origin are symmetrical and compact but those further away are more compressed and elongated at right angles to the direction of development. The sample was also separated in the same chromatographic system, but using linear development on a 10×10 cm plate (Figure 15B).

If the sample is introduced in the mobile-phase stream, then separated bands form concentric rings on the chromatographic plate, as shown in **Figure 16**. This circular chromatogram demonstrates the separation of lipophilic dyes on a silica gel 60 F_{254} high performance TLC pre-coated plate, 10×10 cm (E. Merck) with a mobile phase of hexane–chloroform–NH_3, 70 : 30; the distance of development (from entry position of solvent to eluent front) = 30 mm in a Camag U-chamber.

In the anticircular mode of development the mobile phase enters around the entire periphery of the adsorbent layer which is usually formed as a circle by scraping unwanted adsorbent from a square plate. The samples are applied on an outer circular starting line and development proceeds from the periphery of this circle layer to its centre (Figure 13B). This mode of development can be performed with a Camag anticircular U-chamber, shown in **Figure 17**.

Anticircular chromatography is seldom applied in practice. An example of a chromatogram obtained by this mode of development is given in **Figure 18**. The spots are compact near the origin and elongated in the direction of the mobile-phase migration.

Conclusions

Conventional modes of chromatogram development are often applied in analytical practice for both qualitative and quantitative purposes. The most popular among the modes described is linear development. There are several reasons which contribute to this situation, including a simple operation procedure and low cost and time of analysis per sample. These features will still determine a future use of the modes in the analytical practice of planar chromatography in spite of increasing interest in the application of automated and forced-flow development.

See also: **II/Chromatography: Thin-Layer (Planar):** Instrumentation; Modes of Development: Forced Flow, Over-pressured Layer Chromatography and Centrifugal. **Appendix 2 / Essential Guides to Method Development in Thin-Layer (Planar) Chromatography.**

Further Reading

Geiss F (1987) *Fundamentals of Thin-layer Chromatography* (*Planar Chromatography*). Heidelberg: Hüthig.

Grinberg N (ed.) (1990) *Modern Thin-layer Chromatography*. New York: Marcel Dekker.

Poole CF and Poole SK (1991) *Chromatography Today*. Amsterdam: Elsevier.

Sherma J and Fried B (1996) *Handbook of Thin-layer Chromatography*, 2nd edn. New York: Marcel Dekker.

Zlatkis A and Kaiser RE (1977) *HPTLC High Performance Thin Layer Chromatography*. Amsterdam: Elsevier Science.

Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal

S. Nyiredy, Research Institute for Medicinal Plants, Budakalász, Hungary

Introduction

Forced-flow planar chromatographic separation can be achieved by application of external pressure (over-pressured layer chromatography – OPLC), an electric field, or centrifugal force (rotation planar chromatography – RPC). **Figure 1** shows schematically the superior efficiency of forced-flow techniques by comparing their analytical performance with those of classical thin-layer chromatography (TLC) and high performance thin-layer chromatography (HPTLC). Forced-flow planar chromatography (FFPC) tech-

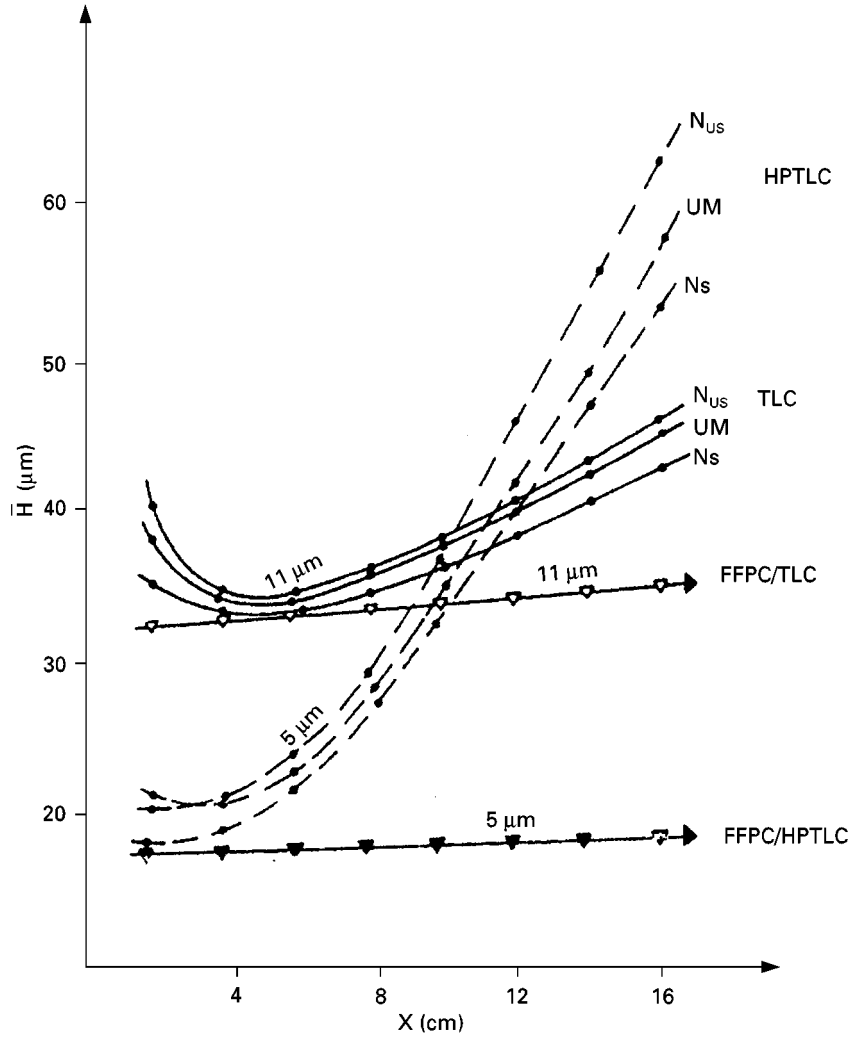

Figure 1 Comparison of the efficiency of analytical TLC and HPTLC chromatographic plates when used with capillary action and forced-flow planar chromatography (FFPC). N_{US}, normal unsaturated chamber; UM, ultramicrochamber; N_{US}, normal saturated chamber.

niques enable the advantage of optimum mobile phase velocity to be exploited over almost the whole separation distance without loss of resolution. This effect is independent of the type of forced flow.

Although FFPC can be started with a dry layer, as in classical TLC, the forced-flow technique also enables fully online separation in which the separation can be started on a stationary phase equilibrated with the mobile phase, as in high performance liquid chromatography (HPLC). The following FFPC combinations of the various offline and online operating steps are feasible:

- Fully offline process: the principal steps, such as sample application, separation, and detection are performed as separate operations
- Offline sample application and online separation and detection
- Online sample application and separation and offline detection
- Fully online process: the principal steps are performed as nonseparate operations.

Overpressured Layer Chromatography

In addition to capillary action, the force driving solvent migration in OPLC is the external pressure. Depending on the desired mobile-phase velocity, operating pressures up to 50 bar can currently be used. In OPLC (**Figure 2**) the vapour phase is completely eliminated; the chromatographic plate is covered with an elastic membrane under external pressure, thus the separation can be performed under controlled conditions. The absence of any vapour space must

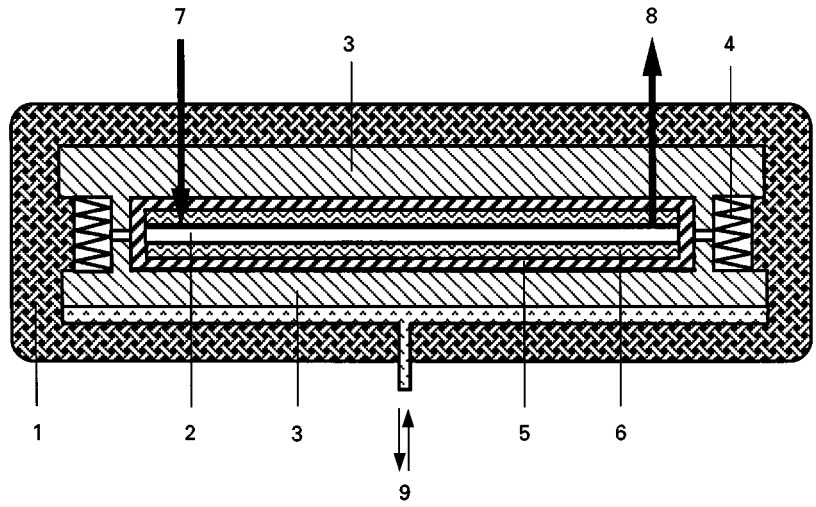

Figure 2 Schematic diagram of online OPLC. 1, Support block; 2, chromatoplate; 3, support plate; 4, spring; 5, casette system for fixing the chromatoplate between two Teflon layers; 6, Teflon layer; 7, Mobile phase inlet; 8, mobile phase outlet; 9, hydraulic system.

be considered in the optimization of the solvent system, especially in connection with the disturbing zone and multifront effect, which are specific features of the absence of a vapour phase (see section entitled 'Elimination of Typical Problems With Use of OPLC').

Principle of Multi-Layer OPLC (ML-OPLC)

OPLC is suitable for the development of several chromatographic plates simultaneously if the plates are specially prepared. With this multi-layer technique, many samples can be separated during a single chromatographic run. By connecting chromatographic plates in parallel (**Figure 3**) more HPTLC plates can be developed simultaneously. By circular OPLC, 360 samples of plant extracts can be separated in 150 s. The rapidity and/or efficiency of the OPLC separation of complex samples can be increased by use of ML-OPLC, in which the same or different types of stationary phase can be used for the development of more chromatographic plates.

Principle of Long-Distance OPLC (LD-OPLC)

Long-distance OPLC is a multi-layer development technique with specially prepared plates. Similar to the preparation of layers for linear OPLC development, all four edges of the chromatographic plates must be impregnated with a polymer suspension. The movement of the eluent with a linear solvent front can be ensured by placing a narrow plastic sheet on the layer or scraping a narrow channel in the sorbent for the solvent inlet. Several plates are placed on top of each other to ensure the long running distance. A slit is cut at the end of the first (upper) chromatographic plate to enable the mobile phase to travel to a second layer. Here the migration continues until the opposite end of the second layer, where solvent flow can be continued to the next adjacent chromatographic plate, or the eluent is led away (**Figure 4**A) if migration is complete. Clearly, on this basis a very long separation distance can be achieved by connecting one plate to another.

Figure 4B shows a typical combination of the same type of chromatographic plate (homoplates). In the arrangement presented, the upper plate has an eluent inlet channel on one side and a slit on the other side for conducting the mobile phase to the next plate. The slit (width approximately 0.1 mm) can be produced by cutting the layer; this enables ready passage of the mobile phase and individual samples without mixing. The cushion of the OPLC instrument is applied to the uppermost layer only, and each plate presses the sorbent layer below. As a consequence of this, glass-backed plates can be used in the lowest position only. The illustrated fully offline separation is complete when the 'α' front (the front of the first solvent in an eluent solvent mixture) of the mobile phase reaches the end of the lowest plate.

The potential of the connected layers can be increased by use of different (hetero) stationary phases during a single development; this is shown in Figure 4C, in which the different sorbents are marked with various shades of gray. The eluate can, furthermore, be led from the lower plate, similarly to the way in which it was led in. This gives the possibility of online detection. For this fully online operating mode all layers placed between the highest and lowest plates must have 1 cm cut from the

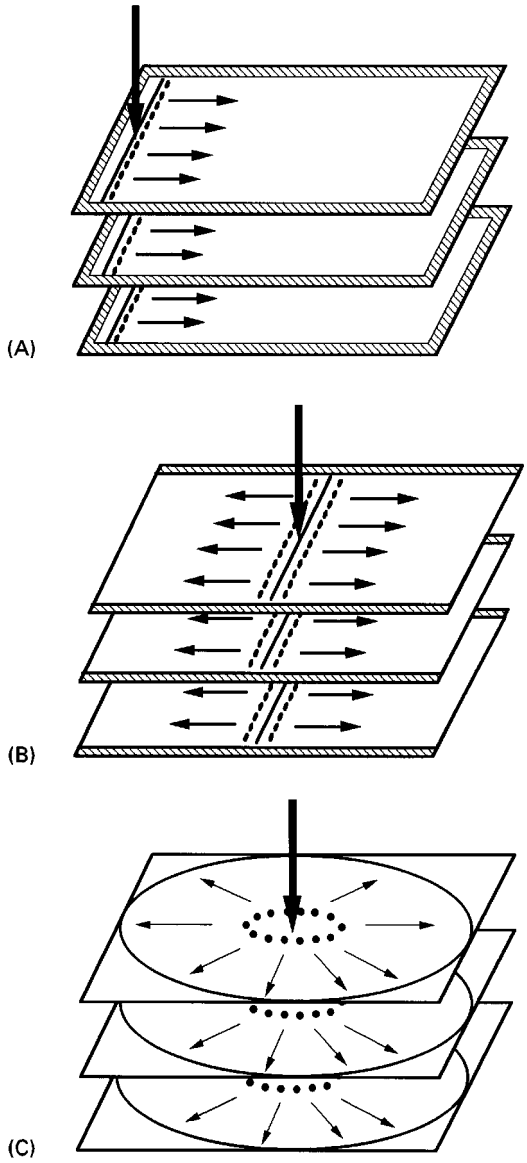

Figure 3 Schematic diagram of multi-layer OPLC (ML-OPLC). (A) Linear one-directional development; (B) linear two-directional development; (C) circular development.

length of the plate, to leave a space for mobile phase outlet.

Analytical OPLC Separations

In OPLC, the most frequent modes of development are linear one- and two-directional (**Figure 5**A,B). Linear OPLC, however, requires a special chromatographic plate sealed along the edge, by impregnation, to prevent the solvent from flowing off the layer.

The advantage of circular development, in which the mobile phase migrates radially from the centre of the plate to the periphery, is well known for the separation of compounds in the lower R_F range, where circular development gives 4–5 times greater resolution. The separating power of circular development is better exploited if the samples are spotted near the centre. As the distance between the mobile-phase inlet and sample application increases, the resolution begins to approach that of linear development. No preparation of the plate is necessary for offline circular OPLC (Figure 5C); for online circular OPLC (Figure 5D) a segment-shaped region must be isolated by removing the surrounding adsorbent and impregnating its edges.

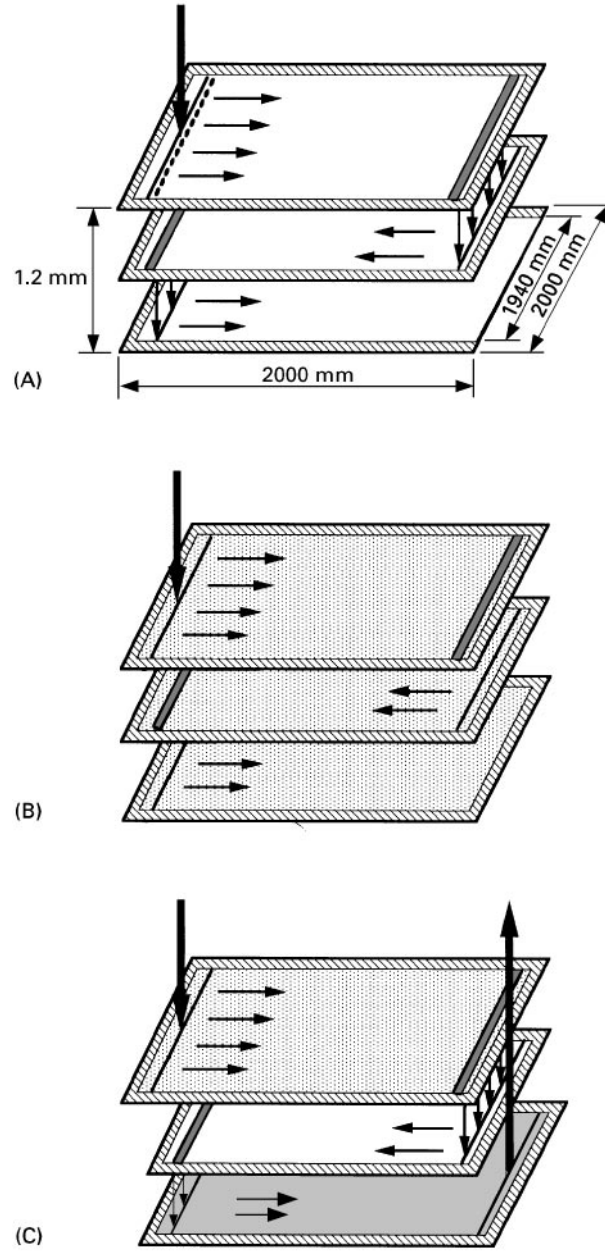

Figure 4 Schematic diagram of long-distance OPLC (LD-OPLC). (A) Principle of the method; (B) fully offline LD-OPLC using homolayers; (C) fully online LD-OPLC using heterolayers.

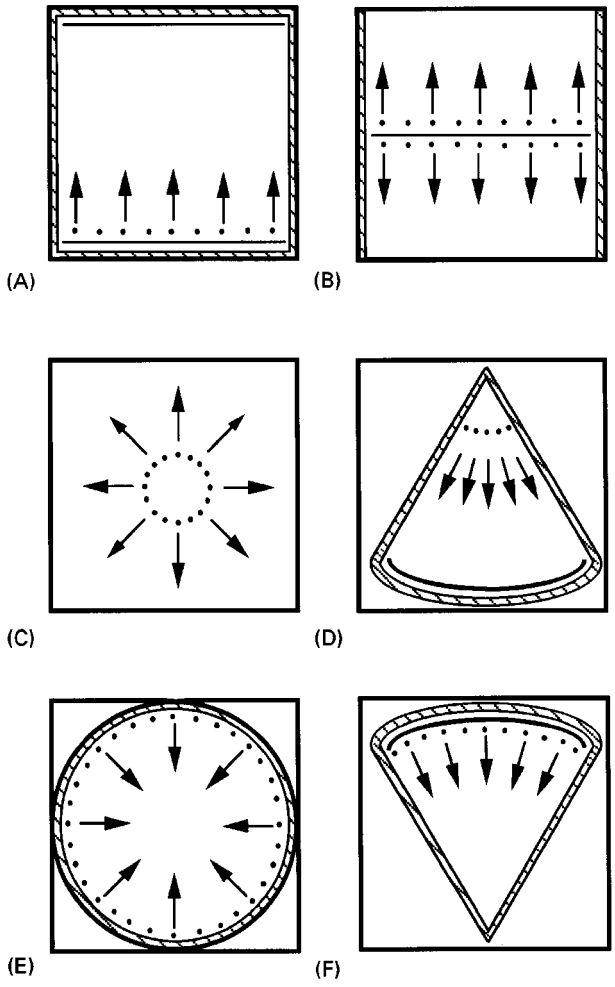

Figure 5 Development modes in analytical OPLC using 20 cm × 20 cm HPTLC chromatographic plates. (A) Linear unidirectional; (B) linear two-directional; (C) circular with 8 cm development distance; (D) circular with 18 cm development distance, or online circular; (E) anticircular; (F) anticircular with 18 cm development distance, or online anticircular.

Conventional offline anticircular separation (Figure 5E) is rather difficult to perform because of the large perimeter of the mobile-phase inlet (ca. 60 cm for a 20 cm × 20 cm plate). Fully offline and online anticircular separations can, however, be performed over a separation distance of 18 cm, after suitable preparation of the plate by isolating a segment of the layer (by scraping) and sealing the isolated segment with polymer suspension (Figure 5F).

In linear OPLC the maximum separation distance is 18 cm for 20 cm × 20 cm chromatographic plates. In offline circular OPLC the maximum separation distance is 10 cm, and only one sample can be analysed. If the distance between the mobile phase inlet and the point of sample application is 2 cm, a separation distance of 8 cm can be achieved; this enables application of more samples.

Micropreparative OPLC Separations

Instrumental methods such as OPLC increase preparation time and costs but also significantly improve efficiency. As a rule of thumb, if the sample contains more than five substances, up to 10 mg of sample can be separated by micropreparative OPLC with linear development on an HPTLC plate. This can be increased five-fold by use of five HPTLC plates and a multi-layer technique; thus preparative amounts can be separated by means of a micropreparative technique. If the sample contains fewer than five substances, the amounts can be increased to 50 mg on a single chromatographic plate. Linear online OPLC is preferable if the structures of compounds to be separated are similar. The circular offline technique can be used if the separation problem is in the lower R_F range.

Probably the most important application of layer switching is in sample clean-up based on a new connection between the layers. A special clean-up effect, sample application and reconcentration, can be achieved simultaneously as shown in **Figure 6**A, in which the upper plate serves for clean-up. Needless to say, these steps can both be performed in fully offline or fully online operating modes, or in freely chosen combinations of different offline and online steps.

The connection illustrated in Figure 6B is an arrangement suitable for a larger amount of complex sample. In this example micropreparative development can be performed on pre-coated fine particle-size analytical plates. The mobile-phase inlet system with the slits is analogous to that for multi-layer development. In the example illustrated, the direction of mobile-phase migration is the same for each pair of plates. The scraped channels are located at the beginning of the upper two layers and the slits are located at the ends of the adsorbent layers. On reaching the end of the first pair of plates the mobile phase passes through to the adjacent pair of layers. Suitable location of channels and slits ensures mobile phase transport through the whole system. The collector channel at the end of the lowest plate leads the eluate to the outlet.

Preparative OPLC Separations

Whether or not the use of OPLC for preparative separation is necessary depends on the kind of sample to be separated. The potential of linear online OPLC on 20 cm × 20 cm plates with a separation distance of 18 cm as a preparative method is considerable. Because the average particle size of pre-coated preparative plates is too large, not all the advantages of this method can yet be realized. Generally, preparative online OPLC can be used for separation of 6–8 compounds in amounts up to 300 mg.

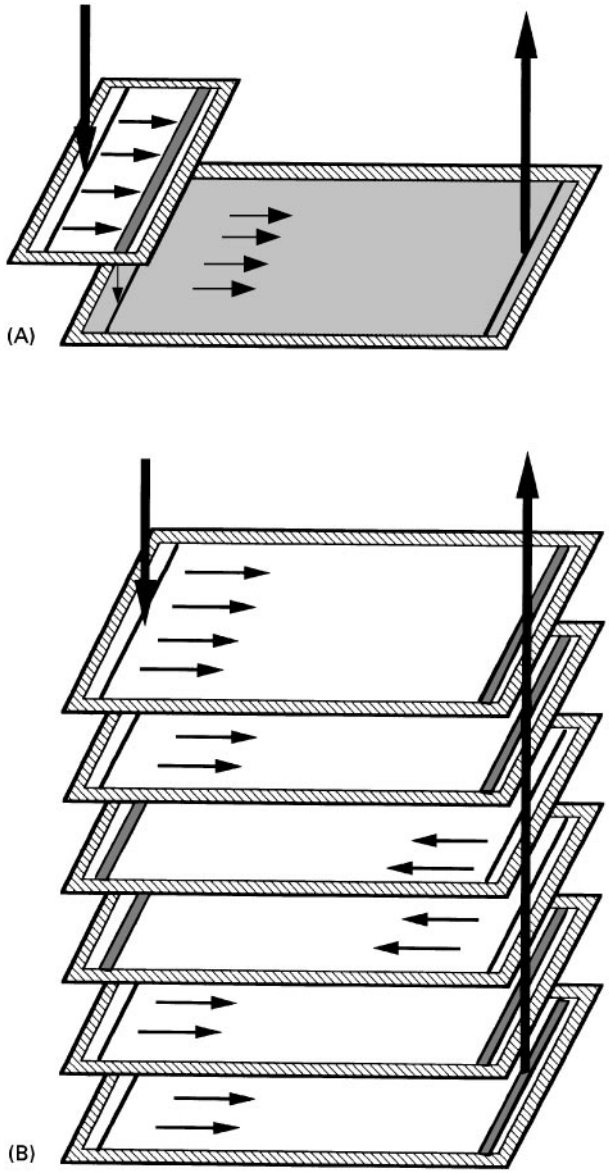

Figure 6 Micropreparative ML-OPLC separations on analytical HPTLC plates. (A) Schematic diagram of cleanup procedure using fully online LD-OPLC; (B) schematic diagram of fully online LD-OPLC for a large amount of a complex mixture.

Elimination of Typical Problems with Use of OPLC

It is of practical importance to summarize the most important distorting effects which arise in OPLC and to describe means of eliminating these problems.

Linear separations require specially prepared chromatographic plates with chamfered edges that are impregnated with a suitable polymer suspension, to prevent solvent leakage at overpressure. For proper preparation of the chromatographic plate, the surface from which the stationary phase has been scratched must be fully cleaned from particles. If this is not achieved, a narrow channel may be formed under the polymer suspension, resulting in faster migration of part of the mobile phase, because of lack of layer resistance; the mobile phase then re-enters further along the plate ('break-in effect' as shown in **Figure 7**A). This reduces the value of the separation, at least at the edge(s) of the layer.

If the area impregnated is too wide, i.e. the edges of the stationary phase covered by the polymer suspension are wider than approximately 1 mm, the 'meniscus effect' can occur (see Figure 7B). As a

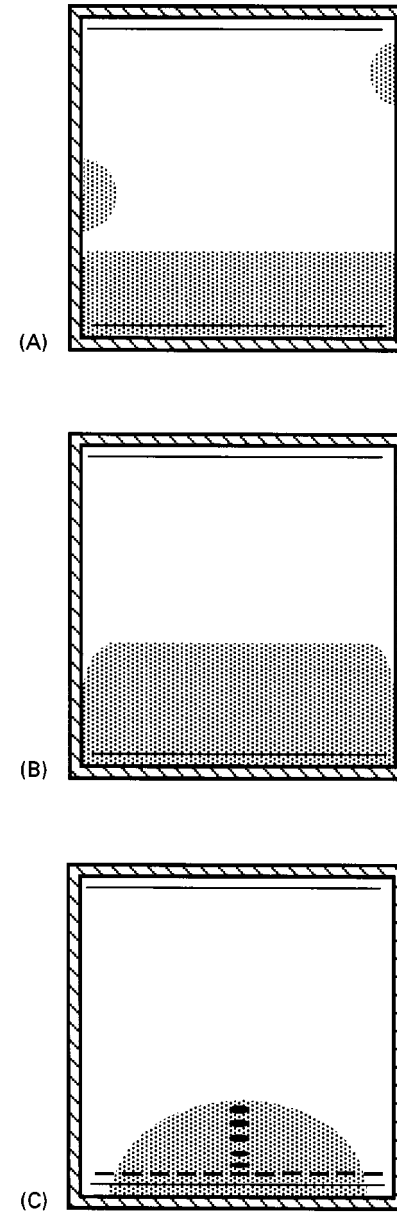

Figure 7 Elimination of typical problems in OPLC. (A) 'Break-in effect' – a consequence of improper impregnation of the chromatographic plate; (B) 'meniscus effect' – a consequence of improper impregnation of the chromatographic plate; (C) lack of the appropriate inlet pressure for linear separation.

consequence of this effect – which occurs either in the concave or convex form, depending on the physical properties of the solvents used – the eluent flows more slowly or more quickly on both edges of the chromatographic plate, again distorting quantitative results.

Before starting the separation with the optimized mobile phase, the mobile phase inlet valve is closed and the eluent pump is started to establish an appropriate solvent pressure. The separation is then started by opening the inlet valve; this ensures the rapid distribution of the mobile phase in the inlet channel necessary for linear migration of the mobile phase. If the inlet pressure is too low and the mobile phase does not fill the inlet channel totally, the start of the separation is similar to that for circular development; the distorted linear separation obtained is shown in Figure 7C. No preparation of the plate is needed for offline circular separations.

If multi-component mobile phases are used in unsaturated TLC, the fronts arising from the components can have a decisive influence on the separation. This effect can be substantial in OPLC; the secondary fronts appear as sharp lines because no vapour phase is present. Compounds of the mixture migrating with one of the fronts form sharp, compact zones whereas tailing or fronting can be observed for compounds migrating directly in front of or behind the α front. With multi-component mobile phases the 'multi-front effect' can appear in two forms. In the first (**Figure 8**A), one or more fronts can occur between compounds to be separated. In the second, all the compounds to be separated migrate behind the lowest front (Figure 8B), and the fronts do not influence the separation. As the position of the fronts is constant, if the chromatographic conditions are constant, possibly undesirable effects of the multi-front effect can be monitored and taken into account by applying the spots or bands stepwise. Thus for linear separations the sample is applied at different distances ($s = 1, 2, ..., n$) from the mobile phase inlet channel (Figure 8C). In circular OPLC the samples are applied at points on concentric circles (or rings) with their centres at the mobile phase inlet (Figure 8D). Quantitative evaluation is usually made more difficult, but not impossible, by the multi-front effect, because the phantom peaks formed at the fronts can be measured densitometrically in the substance-free zones at the sides of the chromatographic plates, and thus the values are taken into account. It must be mentioned that the multi-front effect also has a

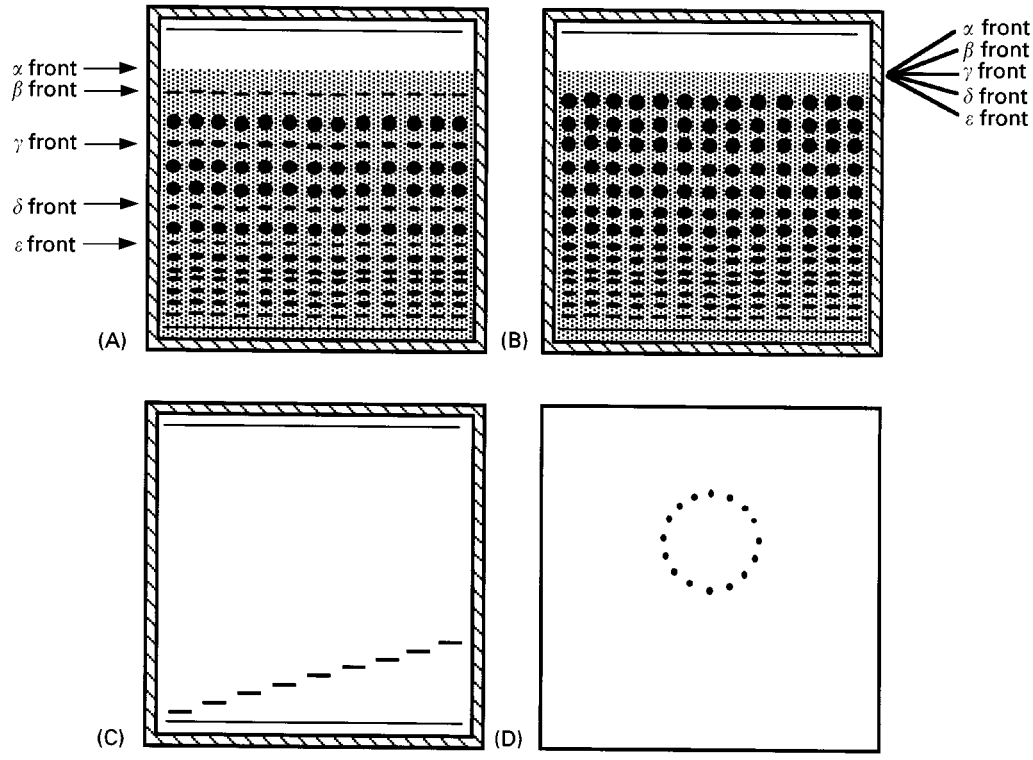

Figure 8 'Multi-front effect' – a consequence of the use of multicomponent mobile phases. (A) The fronts occur between the compounds to be separated; substances migrating with one of the fronts form sharp, compact zones; (B) the compounds to be separated all migrate behind the lowest front, so the fronts do not influence the separation; (C) diagonal application of the samples (as bands) for linear separations to check the place of the different fronts; (D) eccentric application of the samples (as spots) for circular separations to check the place of fhe different fronts.

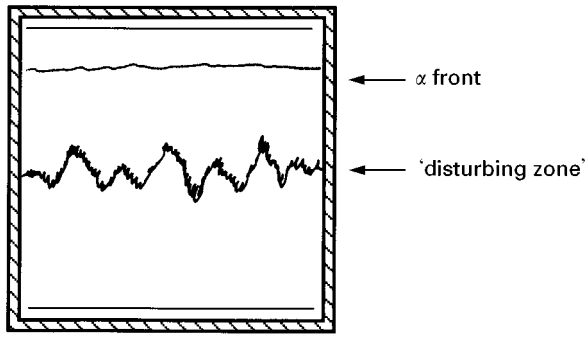

Figure 9 The 'disturbing zone' as a consequence of different air/gas volume ratios adsorbed by the surface of the stationary phase and dissolved in the eluent.

positive effect in preparative separations, because compounds that migrate with α front can be eluted in a very small amount of mobile phase.

If OPLC separation is started with a dry layer, distorted substance zones can sometimes be observed in different R_F ranges, depending on the mobile phase used and the velocity of the mobile phase. This effect appears during the chromatographic process as a zig-zag zone across the width of the plate, perpendicular to the direction of development as a result of the different refractive indices of the solvents in front of and behind this zone. This phenomenon, termed the 'disturbing zone', is depicted in **Figure 9**. The extent of this phenomenon depends on the interrelationship between gas physically bound to the surface of the sorbent and gas molecules dissolved in the mobile phase. Because modification of the location of the 'disturbing zone' is possible within a very narrow range, the only solution to this problem is to conduct a prerun. For separation of nonpolar compounds this can be performed with hexane; for separation of polar substances the prerun can also be performed with hexane or with any component of the mobile phase in which the components are unable to migrate. The selection of this solvent might be considered during optimization of the mobile phase.

Advantages of OPLC

The advantages of the different OPLC methods are summarized as follows:

1. All commercially available chromatographic plates can be used, irrespective of their size and quality; stationary phases prepared from smaller particles can be used without loss of resolution as a result of the overpressure.
2. Mobile phases optimized in unsaturated analytical TLC can be transferred after a suitable prerun.
3. Circular development can be performed without special preparation of the plates; for linear and anticircular development specially prepared plates are necessary.
4. Many samples (up to 72) can be separated rapidly on a single analytical plate and evaluation can be performed densitometrically.
5. Multilayer OPLC is applicable for offline separation of many (up to 360) samples, again with densitometric evaluation.
6. The separation time is relatively short and scale-up for preparative work is simple.
7. All linear separation methods (analytical, micropreparative, preparative) are online; removal of the separated compounds by scraping off the layer is unnecessary.
8. Online determination of a single analytical sample on fine particle-size analytical plates, and online micropreparative and preparative separations can be recorded with a flow through detector.
9. Online preparative separation of 10–500 mg samples can generally be performed in a single chromatographic run.
10. The development distance can be easily increased by use of long-distance OPLC.
11. Combination of different adsorbents can be used in long-distance OPLC so that each part of a complex mixture can be separated on a suitable stationary phase.

Rotation Planar Chromatography

The term 'rotation planar chromatography' (RPC) – irrespective of the type and quality of the stationary phase – embraces analytical, offline micropreparative and online preparative forced-flow planar chromatographic techniques in which the mobile phase migrates mainly with the aid of centrifugal force, but also by capillary action. The centrifugal force drives the mobile phase through the sorbent from the centre to the periphery of the plate. The mobile phase velocity may be varied by adjustment of the speed of rotation.

The different RPC techniques can be classified as normal chamber RPC (N-RPC), micro chamber RPC (M-RPC), ultramicro chamber RPC (U-RPC) and column RPC (C-RPC); the difference lies in the size of the vapour space, an essential criterion in RPC. For analytical separations many samples can be applied. For micropreparative and preparative purposes only one sample is applied as a circle near the centre of the rotating stationary phase. The separations can be performed either in the offline or online mode. In the latter, the separated compounds are eluted from

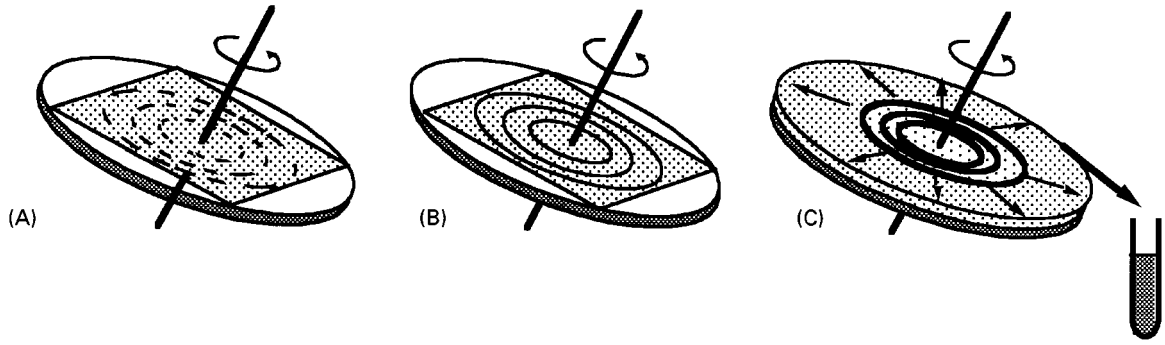

Figure 10 Principles of RPC. (A) Fully offline analytical separation; (B) fully offline micropreparative separation; (C) fully online preparative separation.

the stationary phase by the centrifugal force and collected in a fraction collector (**Figure 10**). All methods can be used for online preparative separations; M-RPC and U-RPC can also be used for analytical and offline micropreparative separations.

Principles of N-RPC, M-RPC and U-RPC

In N-RPC the layer rotates in a stationary chromatographic chamber; in M-RPC – which uses a co-rotating chromatographic chamber – the vapour space is reduced and variable; in U-RPC the layer is placed in the co-rotating chamber from which the vapour space has been almost eliminated. A schematic drawing of preparative M-RPC is shown in **Figure 11**; the layer thickness is approximately 2 mm. When the ultramicrochamber is used, the chromatographic layer is thicker (4 mm); the quartz cover plate is placed directly on the layer so there is almost no vapour space. In N-RPC the quartz plate is removed; this results in a large vapour space.

In all three methods circular development is always used for preparative separations. The sample is applied near the centre of the circular layer, and the mobile phase is forced through the stationary phase from the centre to the outside of the plate (rotor). The separated compounds are eluted from the layer by centrifugal force and collected in a fraction collector. A detector and recorder can be incorporated before the collector.

M-RPC and U-RPC can be used not only for online preparative separations, but also for analytical and offline micropreparative purposes. Excellent resolution is obtained on HPTLC plates.

Principles of S-RPC

For difficult separation problems a special combination of circular and anticircular development can be performed with the sequential rotation planar chromatography (S-RPC). The mobile phase can be introduced onto the plate at any desired place and, time. In S-RPC the solvent application system – a sequential solvent delivery device – works by centrifugal force (circular mode) and with the aid of capillary action against the reduced centrifugal force (anticircular mode). Generally the circular mode is used for the separation, the anticircular mode for pushing the

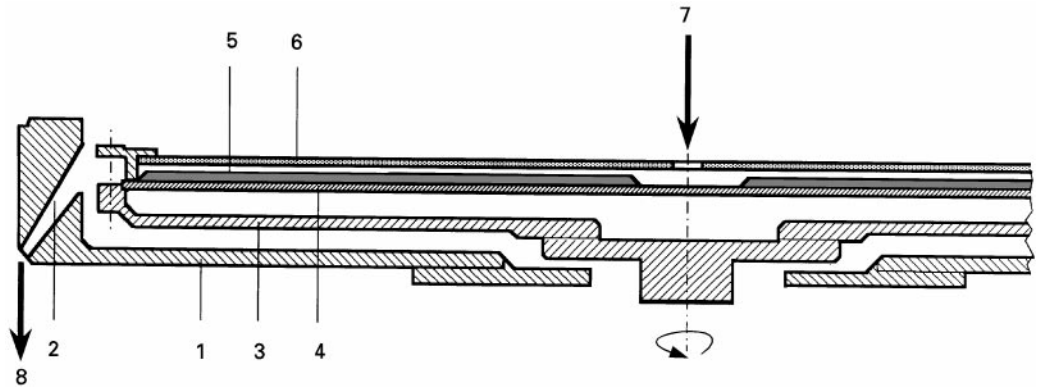

Figure 11 Schematic diagram of preparative M-RPC. 1 = lower part of the stationary chamber, 2 = collector, 3 = motor shaft with the rotating disc, 4 = glass rotor, 5 = stationary phase, 6 = quartz glass cover plate, 7 = mobile phase inlet, 8 = eluent outlet.

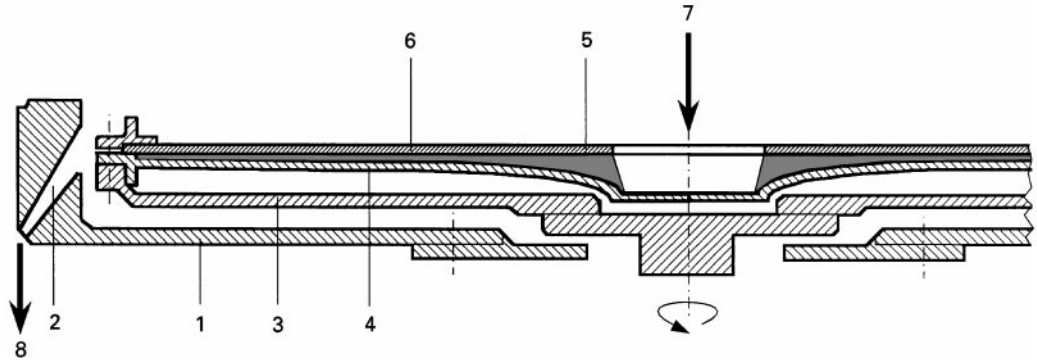

Figure 12 Schematic diagram of preparative C-RPC. 1 = lower part of the stationary chamber, 2 = collector, 3 = motor shaft with the rotating disc, 4 = rotating planar column, 5 = stationary phase, 6 = quartz glass cover plate, 7 = mobile phase inlet, 8 = eluent outlet.

substance zones back to the centre with a strong solvent (e.g. ethanol). After drying the plate with nitrogen at a high rotation speed, the next development with another suitable mobile phase can be started. By combination of the two modes of operation the separation pathway in S-RPC becomes theoretically unlimited.

Principles of C-RPC

In column RPC (see **Figure 12**) there is no vapour space – the stationary phase is placed in a closed circular chamber (column). The volume of stationary phase stays constant along the separation distance and the flow is accelerated linearly by centrifugal force, hence the name 'column' RPC. Because a closed system is used, there is no vapour space and any stationary phase can be used – fine particle size, with or without binder. The rotating planar column has a special geometric design described by eqn [1]

$$h = \frac{K}{(a + br + cr^2)} \qquad [1]$$

where r = radius of the planar column, h = actual height of the planar column at radius r, a, b, c, and K = constants.

This design eliminates the extreme band-broadening which occurs normally in all circular development techniques, and so combines the advantages of both planar and column chromatography.

Analytical RPC Separations

In analytical M-RPC there is a vapour space (1 or 2 mm) between the chromatographic plate and the quartz glass cover plate. In analytical U-RPC a soft crepe rubber sheet is placed underneath the analytical plate so that vapour space between the layer and the quartz cover plate is almost eliminated.

In analytical RPC (M-RPC and U-RPC) three development modes are available and the separation distance and number of samples depend on which mode is used. 20 cm × 20 cm plates can be introduced directly into the instrument. In circular mode (**Figure 13**A) the most commonly used, up to 72 samples can be applied to an HPTLC plate as spots; the separation distance is usually 8 cm. Despite the centrifugal force, the mobile phase direction of flow can be linearized (linear development mode) by scraping channels in the layer (Figure 13B); this reduces the number of samples. The anticircular mode can also be employed with special preparation of the analytical plate (Figure 13C). Although the solvent is delivered

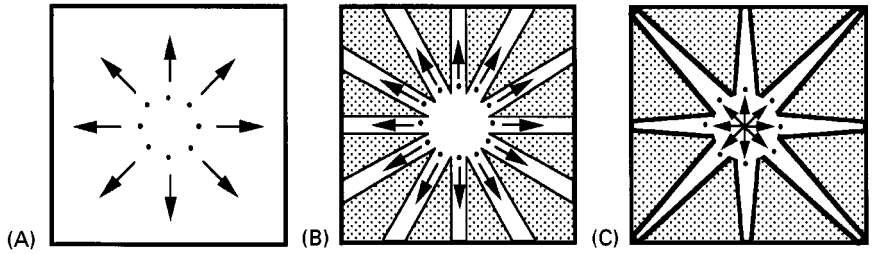

Figure 13 Development modes in analytical RPC on 20 cm × 20 cm HPTLC plates. (A) Circular; (B) linear; (C) anticircular.

at the centre of the plate, in anticircular mode the amount of stationary phase available during development is reduced according to a quadratic relationship by preparation of the layer.

Micropreparative RPC Separations

A mixture of components (5–15 mg) can be applied in the form of a ring near the centre of a single analytical HPTLC plate for isolation of relatively pure compounds by use of U-RPC or M-RPC. The operating process is similar to that used for analytical separations, with the difference that only one sample is applied. Continuous development is possible if a ring of radius 9.8 cm is scraped from the stationary phase, to ensure the regular migration of the mobile phase after it has reached the outskirts of the plate. When the first compounds of interest reach this ring, the separation must be stopped, and either with a stationary rotor or at a rotation speed of 100 rpm the separated components can be scraped out and then the remaining substances can be eluted by the usual procedures, similar to preparative TLC. The separation of ultraviolet (UV)-active compounds can be monitored with a UV lamp during the separation.

Preparative RPC Separations

Because all preparative RPC separations are performed online and no removal of the zones by scratching is necessary, the separation efficiency for the last eluting compounds even increases during the run.

Because M-RPC and U-RPC can be used not only for online preparative separations, but also for analytical purposes, direct scale-up is possible for both analytical methods. From TLC separations using unsaturated or saturated chromatographic tanks, the mobile phase can be transferred via analytical U-RPC and M-RPC to preparative U-RPC and M-RPC, respectively, if the solvent strength and selectivity are kept constant. For scale-up the sample may be applied as a circle to a 20 cm × 20 cm analytical TLC plate and the amount of sample can be increased stepwise in subsequent separations. The adverse effects of different particle sizes and separation distances in the analytical and preparative methods almost cancel each other, so only layer thickness has to be considered in the scale up procedure. The mobile phase flow rate must be adapted to preparative separation, so that the migration of the α front is as fast as in the analytical separation.

Elimination of Typical Problems in RPC

In RPC extra evaporation of the mobile phase occurs owing to the rotation of the chromatographic plate; this can have undesirable effects. In analytical RPC these are the 'surface effect' and the 'effect of the standing front'. The optimum velocity of rotation depends on the particular separation problem. The flow rate is limited by the amount of solvent that can be kept in the layer (layer capacity) without floating over the surface. The greater the amount of solvent applied, the higher the rotation speed must be to keep the mobile phase within the layer. The parameters, rotation speed, perimeter of solvent application and development mode must be considered when setting the pumping speed, otherwise the mobile phase flows over the top of the applied sample and the layer ('surface effect') distorting the separation. A standing front can occur if after a certain time, the well-separated compounds mix back again because the α front becomes stationary owing to the amount of mobile phase evaporating becoming equal to the amount being delivered. When N-RPC is used for preparative purposes, the 'effect of the change of mobile phase composition' is a typical negative effect, which has to be considered during the optimization of the mobile phase.

Advantages of RPC

The advantages of the different RPC methods, can be summarized as follows:

1. Depending on the properties of the compounds to be separated, the effect of the vapour space, and thus the extent of saturation of the chromatographic system, can be selected freely.
2. All commercially available stationary phases can be used, irrespective of their size and quality; smaller particle size stationary phases can be used without loss of resolution because of the centrifugal force.
3. Mobile phases optimized in saturated or unsaturated analytical TLC, or in HPLC, can be transferred to the various RPC methods.
4. All three basic modes of development (circular, linear, and anticircular) and their combinations can be used for analytical separations.
5. For analytical purposes up to 72 samples can be applied to a single analytical plate, and densitometric quantification can be performed *in situ* on the plate.
6. The separation time is relatively short and scaling up to preparative methods is simple.
7. All preparative methods are online, no scratching out of the separated compounds is necessary, and the preparative separation can be recorded with a flow-through detector.
8. Because of the theoretically unlimited separation distance, the separation power can be increased

Table 1 Comparison of the different analytical and preparative FFPC (OPLC and RPC) methods

Method / *Viewpoint*	*OPLC*		*N-RPC*	*M-RPC*		*U-RPC*		*S-RPC*	*C-RPC*
	Analytical	*Preparative*	*Preparative*	*Analytical*	*Preparative*	*Analytical*	*Preparative*	*Preparative*	*Preparative*
Chamber type	Ultra-micro	Ultra-micro	Normal	Micro	Micro	Ultra-micro	Ultra-micro	Normal	Planar column
Plate (column)	TLC/HPTLC pre-coated	Pre-coated	Self-prepared	TLC/HPTLC pre-coated	Self-prepared	TLC/HPTLC pre-coated	Self-prepared	Self-prepared	Self-filled
Stationary phase	All available	Silica	Silica	All available	Silica	All available	Silica	Silica	All available
Layer thickness	0.1, 0.2 mm	0.5–2 mm	1–4 mm	0.1, 0.2 mm	1–3 mm	0.1, 0.2 mm	4 mm	1–4 mm	$\bar{x} = 2.24$ mm
Volume of stationary phase	Constant (increasing)	Constant (increasing)	Increasing	Constant (increasing)	Increasing	Constant (increasing)	Increasing	Increasing and decreasing	Constant
Particle size of stationary phase	5,11 μm	5 μm $< x <$ 25 μm	15 μm	5,11 μm	15 μm	5–11 μm	15 μm	15 μm	5 μm
Separation distance	18 (90) cm	18 cm	10 cm	8(11) cm	10 cm	8(11) cm	10 cm	Unlimited	9 cm
Separation mode	Circular, linear, (anticircular)	Linear (circular)	Circular	Circular, linear, (anticircular)	Circular	Circular, linear, (anticircular)	Circular	Circular and anticircular	Linear
Observation	Not possible	Not possible	Coloured and UV active substances can be observed during the chromatographic process						
Detection	Offline, online	Online	Online	Offline	Online	Offline	Online	Online	Online
Sample number	Max. 360	1	1	max. 72	max. 72	max. 72	1	1	1
Amount of sample	ng–μg	50–500 mg	50–500 mg	ng–μg	50–500 mg	ng–μg	50–500 mg	50–500 mg	50–500 mg

significantly by employing the sequential technique.

9. On line preparative separation of 50–500 mg samples can generally be applied in a single chromatographic run.

Comparison and Outlook of FFPC Methods

The various OPLC and RPC techniques are compared in **Table 1**. Study of the data shows that OPLC is an excellent technique for analytical separations and that RPC is more ideally suited as a preparative method for isolation of compounds from biological matrices.

The advantage of combining online and offline separations and two-dimensional development can also be exploited in OPLC. The advantage of multiple development methods is the possibility of analytical RPC separations. A realistic means of increasing the efficiency of the planar chromatography of complex samples is the use of long-distance OPLC for analytical separations and sequential RPC for preparative purposes. Working with multi-layer OPLC, the rapidity of the separation can increase significantly, providing new vistas in screening and genetic work.

FFPC techniques will open up a new field of planar chromatography, particularly in the separation of complex samples. It is expected that future research will concentrate on the positive effects (applied pressure in OPLC and higher centrifugal force in RPC) of forced flow. As a consequence, smaller particle size, narrower distribution range, and spherical stationary phases will be needed to achieve maximum resolution.

See also: **II/Chromatography: Thin-Layer (Planar):** Instrumentation; Modes of Development: Conventional; Preparative Thin-Layer (Planar) Chromatography; Theory of Thin-Layer (Planar) Chromatography. **Appendix 2/ Essential Guides to Method Development in Thin-Layer (Planar) Chromatography.**

Further Reading

Botz L, Nyiredy Sz and Sticher O (1990) The principles of long distance OPLC, a new multi-layer development technique. *Journal of Planar Chromatography* 3: 352–354.

Geiss F (1987) *Fundamentals of Thin Layer Chromatography* (*Planar Chromatography*). Heidelberg: Hüthig.

Nurok D, Frost MC, Pritchard CL and Chenoweth DM (1998) The performance of planar chromatography using electroosmotic flow. *Journal of Planar Chromatography* 11: 244–246.

Nyiredy Sz (1992) Planar chromatography. In: Heftmann E (ed.) *Chromatography*, 5th edn, pp. A109- 150. Amsterdam: Elsevier.

Nyiredy Sz and Fatér Zs (1994) The elimination of typical problems associated overpressured layer chromatography. *Journal of Planar Chromatography* 7: 329–333.

Nyiredy Sz, Botz L and Sticher O (1989) ROTACHROM®: A new instrument for rotation planar chromatography (RPC). *Journal of Planar Chromatography* 2: 53–61.

Nyiredy Sz, Botz L and Sticher O (1990) Analysis and isolation of natural products using the ROTACHROM® rotation planar chromatograph. *American Biotechnology Laboratory* 8: 9.

Sherma J and Fried B (1995) *Handbook of Thin-Layer Chromatography*. New York: Dekker.

Tyihák E and Mincsovics E (1988) Forced-flow planar liquid chromatographic techniques. *Journal of Planar Chromatography* 1: 6–9.

Tyihák E, Mincsovics E and Kalász H (1979) New planar liquid chromatographic technique: overpressured thin-layer chromatography. *Journal of Chromatography* 174: 75–81.

Tyihák E, Mincsovics E and Székely TJ (1989) Overpressured multi-layer chromatography. *Journal of Chromatography* 471: 375–387.

Preparative Thin-Layer (Planar) Chromatography

S. Nyiredy, Research Institute for Medicinal Plants, Budakalász, Hungary

Introduction

Preparative planar (thin-layer) chromatography (PPC) is a liquid chromatographic technique performed with the aim of isolating compounds, in amounts of 10–1000 mg, for structure elucidation (mass spectrometry (MS), nuclear magnetic resonance (NMR), Infrared (IR), ultraviolet (UV) etc.), for various other analytical purposes, or for determination of biological activity. PPC is a valuable method of sample purification for preparative purposes and isolation. The scope for modifying operating parameters such as the vapour space, development mode and for offline sample application is enormous in planar chromatography.

In classical PPC the mobile phase migrates by capillary action, whereas if forced-flow PPC (FFPPC) is