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Planar chromatography: current status and future perspectives in pharmaceutical analysis (Short Review) — II. Special techniques and future perspectives in planar chromatography*

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Abstract: The state of the art of various special analytical planar chromatographic methods is summarized especially for forced-flow planar chromatography (FFPC), overpressured-layer chromatography (OPLC) and rotation planar chromatography (RPC) as well as for the automated multiple development (AMD) technique. The connection between analytical planar and column liquid chromatographic methods and the identification of separated compounds with chromatographic and spectroscopic data are summarized. Some aspects of future perspectives, such as parallel connected multi-layer FFPC and long distance OPLC (LD-OPLC) are given. A combination of OPLC with the AMD method is predicted as the method of the future. Strategies using FFPC techniques are suggested in the form of a flow chart.

Keywords: *Planar chromatography; FFPC; OPLC; LD-OPLC; RPC; AMD technique.*

Special Separation Techniques

In planar chromatography the mobile phase can migrate through the stationary phase by capillary action or under the influence of forced flow. The category of forced-flow planar chromatography (FFPC) includes all methods where the mobile phase migrates not only with the capillary action but also with forced flow. Three basic FFPC methods have been used. Forced flow is achieved by pressure, by an electric field or by centrifugal force [1]. In recent years the application of external pressure for overpressured-layer chromatography (OPLC) and centrifugal force for the different types of rotation planar chromatography (RPC) have been strongly developed. Figure 1 schematically demonstrates the enhanced efficiency of FFPC techniques by comparing their analytical properties with those of classical thin-layer chromatography (TLC) and high-performance TLC (HPTLC).

With FFPC techniques the advantage of the optimal mobile phase velocity can be exploited practically over the whole separation of the layer thickness (analytical or preparative plates) and the type of forced flow applied [2].

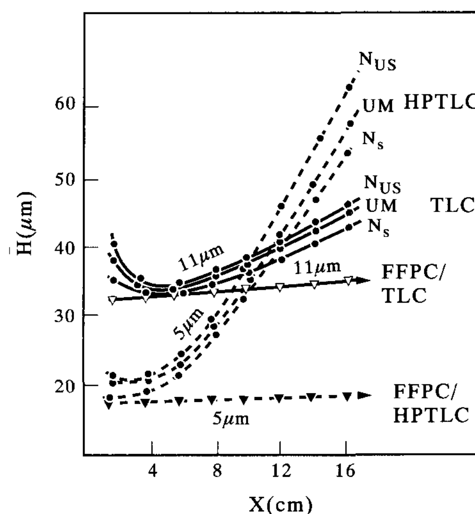

Figure 1
Comparison of capillary flow-controlled and forced-flow planar chromatography methods for different plates (TLC, HPTLC) and chamber types. X = developing distance from the start to the solvent front, $\bar{H}$ = plate height, N_{US} = normal, unsaturated chamber, UM = ultramicro-chamber, N_S = normal, saturated chamber.

In FFPC separation may be started with a dry layer, as in classical TLC (fully off-line separation), but the closed system also permits fully on-line separation to be achieved in which

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separation can be started with the mobile-phase equilibrated stationary-phase system, as in high-performance liquid chromatography (HPLC). Therefore the fully on-line FFPC is a planar variation of HPLC [3].

Overpressured-layer chromatography

Apart from capillary action the driving force for solvent migration in OPLC is external pressure [4–7]. Depending on the desired mobile phase velocity, low (2–5 bar), medium (10–30 bar), and high (50–100 bar) operating pressures can be used [8]. With this type of forced flow, the advantage of optimal mobile-phase velocity can be exploited over practically the whole separation distance without loss of resolution. In OPLC the vapour phase is completely eliminated, the chromatoplate being covered with an elastic membrane under external pressure; thus, separation can be carried out under controlled conditions. Separation may be started with a dry layer, as in classical TLC, but the closed system also permits fully on-line separation to be achieved, where the separation can be started with the mobile-phase equilibrated stationary-phase system, as in HPLC.

The chambers used for OPLC separations are unsaturated S-chambers, theoretically and practically devoid of any vapour space. This must be considered in the optimization of the solvent system, especially in connection with the disturbing zone [9] and multifront effect [10], which are specific features of the absence of a vapour phase.

OPLC separations can be performed on two commercially available instruments, the Chrompres 10 and Chrompres 25 (Laboratory Instruments Works Co. Ltd, Budapest, Hungary). The first type offers different separation distances (18 and 36 cm) in the on-line mode and, in addition, off-line circular development. Chrompres 25 accommodates a higher cushion pressure (25 bar) than Chrompres 10. This allows the use of more viscous mobile phases and/or higher mobile phase velocities, in the linear development mode.

Linear separations require specially prepared plates, with edges that are chambered off and impregnated with a suitable polymer suspension, in order to prevent solvent leakage at overpressure. No preparation of the plate is needed for off-line circular separation for a maximum separation distance of 9 cm. At higher development distances for circular as well as for anticircular operating modes the plates have to be specially prepared [10].

A new, radial version of OPLC, high-pressure planar liquid chromatography (HPPLC) has been developed by Kaiser and Rieder [11]. In this method the sample application and mobile phase inlet are identical, i.e. the centre of the chromatoplate. Only one sample is generally chromatographed on a 10 × 10 cm HPTLC plate at approximately 30 bar external pressure. The main fields of use of HPPLC are mobile phase transfer and direct coupling with HPLC because the k' data can be transferred between the two methods with an accuracy of 2%; in addition fast routine single sample analysis can be carried out with high accuracy, including the calibration [12, 13].

Rotation planar chromatography

In RPC the driving force for solvent migration is centrifugal force in addition to capillary action. The samples are applied to the rotating stationary phase near to the centre. The centrifugal force drives the mobile phase through the sorbent from the centre to the periphery of the plate. For analytical purposes, up to 72 samples can be applied and quantification can be carried out *in situ* on the plate. For micropreparative and preparative purposes only one sample is applied as a circle; the separations can be carried out either in the off-line or the on-line mode. In the latter, the separated compounds are eluted from the stationary phase by centrifugal force and collected in a fraction collector [14, 15].

The main difference between the chamber types used in RPC lies in the size of the vapour space, which is an essential criterion in RPC [15]. Therefore an additional symbol is used to indicate the vapour space [normal chamber, microchamber, ultramicrochamber, and column RPC (N-RPC, M-RPC, U-RPC and C-RPC, respectively)].

In N-chamber RPC the layer rotates in a stationary N-chamber, where the vapour space is extremely large. Owing to extensive evaporation, this chamber is practically unsaturated. The M- and U-chambers in RPC belong to the S-chamber type, the difference between these two chambers being that the former is saturated, while the latter is unsaturated. Since in M-chamber RPC the chromatoplate rotates together with the small chromatographic chamber in which the

distance between the layer and the lid of the chamber is less than 2 mm, the vapour space is rapidly saturated. In the case of the ultramicro-chamber the lid of the rotating chamber is placed directly on the chromatoplate [3] so that practically no vapour space exists.

Two instruments were developed for RPC: the Chromatotron Model 7924 (Harrison, Palo Alto, CA, USA), and the Rotachrom™ Model P rotation planar chromatograph (Petazon, Zug, Switzerland).

Automated multiple development

In conventional multiple development, the chromatogram is run repeatedly over the entire migration distance with intermediate drying, whereas automated multiple development (AMD) involves several stages (between 15 and 30) of different lengths [16–18]. The distance covered per step is larger than its predecessor by a constant increment, which is typically 1–3 mm per stage. Between stages the plate is vacuum dried and thereby activated for the next run. The systematic change of solvent strength from polar (methanol) to apolar (hexane) solvent intensifies the concentrating effect and is responsible for a peak width that is independent of migration distance and width of the starting zone [19]. That means that relatively large samples can be applied, which can be important for pharmaceutical trace analysis.

A typical universal gradient of 25 steps is depicted in Fig. 2. The upper part shows the generally used gradient which commences with methanol and ends with hexane and uses dichloromethane as the intermediate solvent [20]. On the lower part of Fig. 2 the changes in solvent strength is shown during the 25 chromatographic steps.

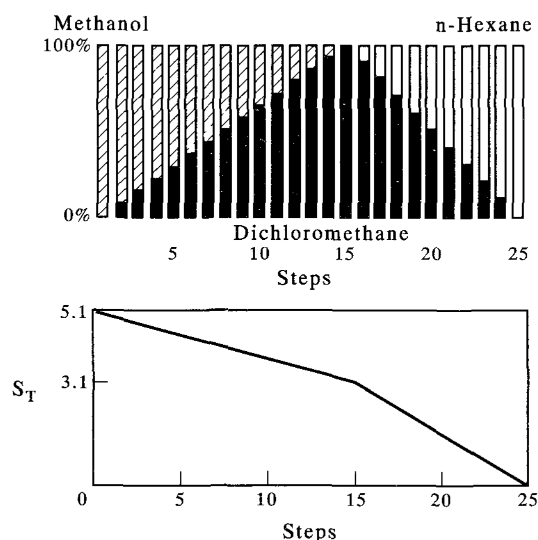

Figure 2
A typical universal gradient for the AMD technique. S_T = solvent strength.

A main advantage of the method is that the sharply focused zones migrate different distances according to their polarity. When the solvent strength of the gradient is low enough the compounds occupy their final position in the chromatoplate. Owing to the systematically decreasing solvent strength, diffusion of the compounds is highly reduced.

The central component of the AMD equipment (Camag, Muttenz, Switzerland) is a closed N-chamber with connections for feeding and withdrawing developing solvents, and for pumping a gas phase in and out. The mobile phase system comprises up to six reservoir bottles containing the neat solvents which are passed via to a gradient mixer [19]. The gas phase is made up externally by passing nitrogen through the wash bottle into the reservoir, from where it is pumped into the chromatographic chamber at the appropriate time.

Connection between planar and column liquid chromatographic methods

The main problems in planar chromatography are poor reproducibility and the transfer of mobile phase between the various chromatographic methods, due to the change in the chamber saturation [21]. Recently one of the authors [22] proposed a characterization method for chamber saturation. Based on this system, good reproducibility and mobile phase transfer between the analytical planar and column chromatographic methods can be achieved. The following scheme (Fig. 3) shows the transfer possibilities of the mobile phase between the various analytical liquid chromatographic methods, where the thick lines indicate direct transfers. The thin lines indicate other transfers which are also possible; in general, the selectivity has to be changed.

The advantage of this procedure is that the mobile phase of the TLC separation where the substance zones are distributed over the whole R_f range can be transferred to HPLC without limitations.

It is interesting that because OPLC may be used as an equilibrated or nonequilibrated planar column system and all mobile phase fronts may be seen, it can be applied as a pilot

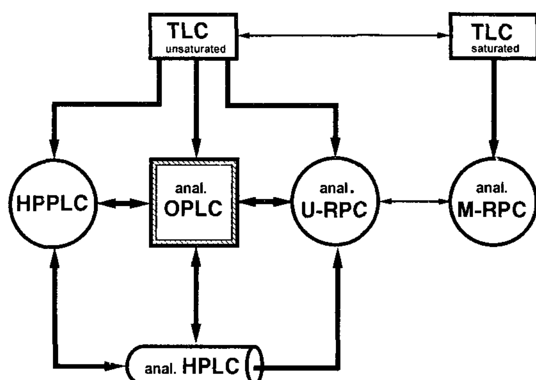

Figure 3
Mobile phase transfer possibilities between planar and column liquid chromatographic methods.

method for preparative column liquid chromatography. Depending on the results of the analytical OPLC separation, four possibilities exist for this purpose [2, 3]. A commonly used method is to equilibrate the dry-filled column with a solvent in which the substances to be separated do not migrate and which was used for the prerun in analytical OPLC. The separation is then started with the optimized TLC mobile phase where the substances are distributed over the whole R_f range.

Comparison of special analytical techniques used in planar chromatography

The properties of various special techniques are summarized in Table 1.

Identification with off-line UV–vis spectra

Apart from chromatographic data, analytical methods that can be used for the analysis of a chromatoplate include visual inspection, UV–vis spectrophotometry, fluorescence spectrophotometry, optical and electron microscopy techniques, Auger, reflectance IR spectroscopy, radioimaging methods, near-IR analyses, and mass spectrometry in various forms including secondary-ion mass spectrometry, fast-atom bombardment, and laser desorption ionization [3]. It should be noted that chromatographic retention data are not sufficient for correct identification; at least one spectroscopic method is necessary to make a valid statement of identity.

If the retention data (R_f, hR_f, R_m or R_x values) of the compound to be identified are identical with those of the reference substances in three different solvent systems but the same stationary phase or with the same solvent system but three different types of stationary phase, the two compounds can be regarded as identical with a good probability. The probability can be most easily increased by off line (*in situ*) UV and/or visible spectra and after colour reaction with off-line visible spectra. A typical identification with off-line spectra in the UV and visible range [Fig. 4(b), (c)] as well as after postchromatographic derivatization is shown in Fig. 4(d).

Future Perspectives

Parallel connected multi-layer FFPC

Tyihák *et al.* [23] found that OPLC is suitable for the development of several chromatoplates simultaneously if the plates are specially prepared. With this multilayer technique [see Fig. 5(a)] a great number of samples can be separated during a single chromatographic run. In this version of OPLC the same or different types of stationary phases can be used for the simultaneous development of several chromatoplates. This version is not only excellent for rapid off-line analytical OPLC but also suitable for RPC separations. The efficiency of the method allows many special prepared chromatoplates to be developed simultaneously for the separation of more hundred samples during a single chromatographic run (Fig. 5).

Parallel connected multilayer FFPC can be carried out for linear and circular OPLC as well as for circular RPC for analytical and micropreparative purposes.

Serial connected multi-layer FFPC (long distance OPLC)

Recently Botz *et al.* [24, 25] proposed a novel OPLC development technique in which the separation distance can be freely increased due to the special arrangement of the chromatoplates. This category of multilayer FFPC involves the serial connection of chromatoplates, and is known as long-distance OPLC [see Fig. 5(b)], in which the efficiency of separation is increased significantly. When applying this technique it is also possible to use different stationary phases in planar chromatography during a single development.

For long-distance (serial connected) OPLC in the linear development mode specially prepared plates are necessary. Similar to the preparation of layers for linear OPLC development, the edges of the chromatoplates must

Table 1
Comparison of various special analytical techniques used in planar chromatography

Properties	OPLC	HPPLC	U-RPC	M-RPC	AMD
Operation	difficult	easy	easy	moderately difficult	easy
Sensitivity to experimental conditions	very sensitive	not sensitive	not sensitive	sensitive	not sensitive
Mobile phase selection	problems occur by solvent demixing	problems occur by solvent demixing	practically no problems	standing front effect, overflow effect	limited
Separation efficiency	high	depends on R_f range	good	good	high
Number of separation variables for phase separation optimization	moderate	moderate	moderate	moderate	high
Quantitation	acceptable	high accuracy and precision	acceptable	acceptable	excellent reproducibility
Special features					
sample application	on-line possibility	on-line possibility	off-line	off-line defined	off-line defined
vapour space	absent	absent	practically absent		
detection	on-line possibility	on-line possibility	densitometry; separation visible	densitometry; separation visible	densitometry
separation distance	long-distance possibility (up to 100 cm)	up to 5 cm	up to 10 cm	up to 10 cm	up to 150 cm
temperature	temp. control and temp. programming	no problems	no problems	difficult	probably difficult
number of samples	up to 360	1 (up to 24)	up to 72	up to 72	up to 24
flow rate	depending on overpressure	depending on overpressure	depending on centrifugal force	depending on centrifugal force	capillary force
development mode	linear, circular, anti-circular, two-dimensional and directional	circular (anticircular)	circular (anticircular, linear)	circular (anticircular, linear)	linear
multi-development approach	usable	no	no	no	no
multi-separation approach	usable	no	no	no	no

Figure 4
Identification of anthraquinone aglycones with UV–vis spectra. (a) densitogram of the separation; (b) UV spectra of the separated compounds; (c) visible spectra of the separated compounds; (d) visible spectra of the separated compounds after post-chromatographic derivatization. 1, rhein; 2, aloe emodin; 3, emodin; 4, physcione; 5, chrysophanic acid.

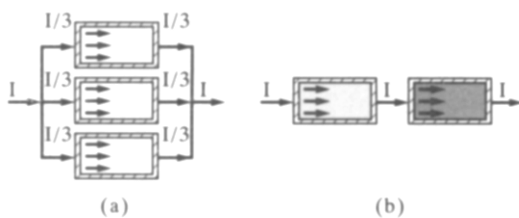

Figure 5
Principle of multi-layer FFPC. (a) parallel connected OPLC. (b) Serial connected OPLC (long-distance OPLC).

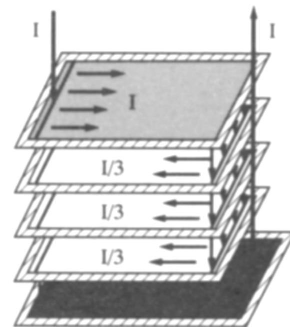

Figure 6
A combination of parallel and serial connected cromatoplates for multi-layer OPLC separation.

be impregnated. 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 run distance. The end of the first top chromatoplate has a slit-like performation to permit the mobile phase to travel to a second layer where the migration continues to the opposite end of the second layer where either the travelling can be continued to the next chromatoplate or the eluent can be led away. Also it is even possible that migration is complete. On this basis a very long separation distance can be achieved by adding one plate to another. In consequence of the layer preparation, glass plates can only be used in the lowest position [25].

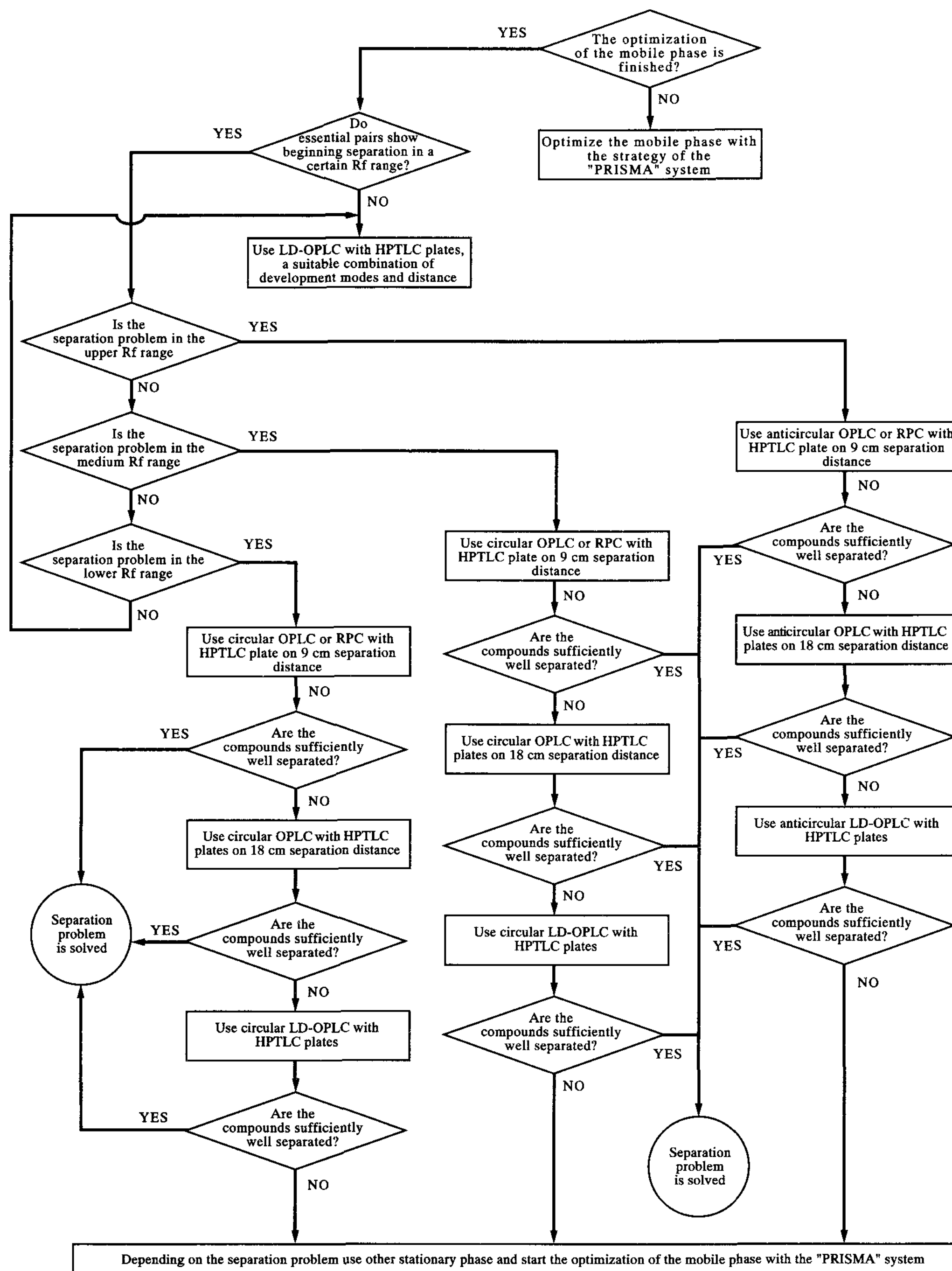

Figure 7
Selection of FFPC methods in the form of a flow chart.

In this technique the upper plate has an eluent inlet channel on one-side and a slit on the other side to enable the mobile phase to travel to the next plate. The slit (width about 0.1 mm) can be located on the layers using sharp-cutting, permitting a good passage of the mobile phase and individual samples without any mixing [25]. The cushion of the OPLC instrument is applied to the uppermost layer only and each plate presses the sorbent layer below.

The potential of the serial connection of layers can be increased using different (hetero) stationary phases during one development. Furthermore, the eluate can be led out from the lower plate, similar to the way in which it is led in. This gives the possibility for the fully on-line mode to be operated.

From the various possibilities of combinations of multi-layer FFPC techniques in Fig. 6 a combination of parallel and serial connected chromatoplates is shown. In this arrangement of the plates the middle three plates are connected in parallel; the lowest, the middle three and the top are arranged linearly. In Fig. 6 also the type of sorbent materials are varied; the different stationary phases are marked in various shades of grey. Note that at this connection the local mobile phase velocity is different between the parallel and the serial connected chromatoplates.

The selection of FFPC methods (RPC, OPLC, LD-OPLC) at different separation distances depending on the separation problem is summarized in Fig. 7.

Combination of LD-OPLC with AMD

A very realistic possibility of increasing the efficiency and speed of planar chromatographic separation would be the combination of LD-OPLC with AMD. Using this method all advantages of the forced-flow technique could be combined with multi-separation and the multi-development mode (circular, anticircular) with off-line and on-line detection as well as the AMD separation method.

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