The bridge between TLC and HPLC: overpressured layer chromatography (OPLC)

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An account of the basic principle of overpressured layer chromatography (OPLC) is given, followed by a description of the commercially available instrument, operating and development modes, as well as special techniques and how to eliminate typical problems arising during the use of OPLC. A comparison of fully off-line and on-line OPLC separations and the correlation between OPLC and HPLC retention data are summarized. Although this article is not intended as a comprehensive review, reference is made to a number of papers and reviews enabling the reader to acquire a deeper knowledge of the topic.

Keywords: Overpressured layer chromatography (OPLC); On-line mode; Off-line mode; Development modes; Mobile phase transfer

1. Status and principle of OPLC

Liquid chromatography (LC) can be classified into column and planar chromatographic techniques. In the former category, besides the many preparative techniques such as open-column chromatography, vacuum liquid chromatography, flash chromatography (FC), low-pressure liquid chromatography (LPLC), medium-pressure liquid chromatography (MPLC) and high-performance (high-pressure) liquid chromatography (HPLC) only the last can be used for analytical separations. In the planar methods – all of which can be used for analytical, micropreparative and preparative purposes – the solvent can migrate through the stationary phase by capillary action, as in thin layer chromatography (TLC) and high-performance TLC (HPTLC) or under the influence of forced flow [1]. Forced flow can be achieved either by use of centrifugal force, as in rotation planar chromatography [2–4], by application of an electric field, as in high-speed TLC or electroplanar chromatography [5,6], or by application of external pressure, as in overpressured layer chromatography (OPLC) [7–9].

Of the three forced-flow planar chromatography techniques, the novel one, OPLC, was first described by Tyihák, Mincsovics and Kalász [7]. In addition to capillary action, the solvent migration is carried out through the pump which delivers the mobile phase. For this technique a specially prepared TLC plate is covered by a flexible inert sheet which is subjected to overpressure and the mobile phase is then pumped through the sorbent layer. In OPLC the vapor phase is completely eliminated, the chromatographic plate being covered with an elastic membrane under external pressure, and the separation can thus be performed under controlled conditions. The method is a hybrid between conventional TLC and HPLC and incorporates several of the attractive features of each technique [9]. Fig. 1 shows schematically the superior efficiency of the OPLC techniques by comparing their analytical performance with those of classical TLC and HPTLC. OPLC techniques enable the advantage of optimum mobile phase velocity to be exploited over almost the whole separation distance without loss of resolution. This configuration enables mobile phase to be pumped through the layer, and results in a substantially shorter analysis time and higher efficiency compared with conventional TLC/HPTLC. OPLC increases preparation time and costs but also significantly improves efficiency [10].

2. The OPLC instrument

Depending on the desired mobile phase velocity, operating pressures up to 50 bar can currently be used. In the commercial design a Teflon™ cover sheet is used in conjunction with a hydraulic press

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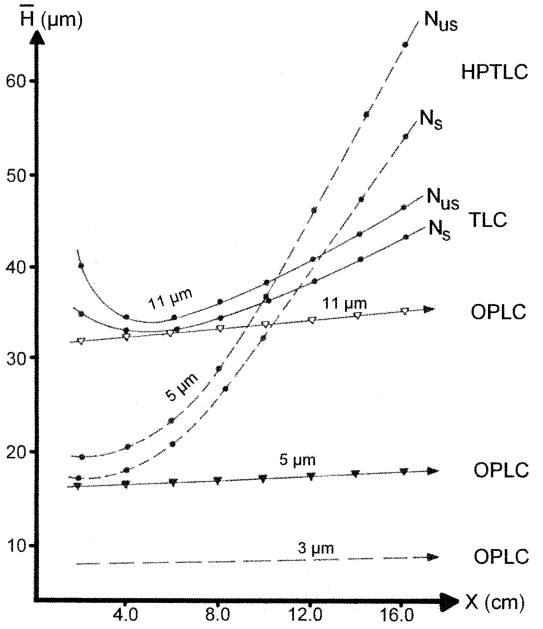

Fig. 1. Comparison of the efficiency of analytical TLC and HPTLC chromatographic plates when used with capillary action and overpressured layer chromatography (OPLC). N_{US}, normal unsaturated chamber; N_{US}, normal saturated chamber.

for supplying the overpressure. The design of the commercially available third generation is a cassette-type apparatus (Fig. 2a) where the prepared TLC plate – as shown in Fig. 2b – is placed in a holder which is inserted through a slot into the equipment. Before performing a separation in the linear mode, it is necessary to seal the edges of the chromatoplate in order to prevent the mobile phase from running off the plate. About 2 mm of the layer is scraped from the edges of the plate, and the exposed backing and 2 mm of the sorbent layer are coated with a suitable polymer [10]. Chromatoplates with sealed edges are commercially available.

An overpressure of up to 50 bar can be used, which allows faster flow rates than in the earlier designs, which were limited to pressures of 10 and 25 bar, respectively. The higher operating pressure is important as it allows a high flow rate even with small-particle layers, like HPTLC. Moreover, higher pressure results in an increase in efficiency, even when the flow rate is held constant. The improvement in efficiency is due both to the flexible membrane forming a better seal with the layer surface and to a slight compression of the layer, which results in a reduction in the interstitial porosity. Reduced plate heights of about 2.3 times less have been reported for layers consisting of spherical 3 µm particles at an overpressure of 50 bar [11]. This is about half the value for HPTLC layers used for conventional TLC. The actual plate heights were in the range 10–30 µm for layers consisting of 5 µm particles, and 6–15 µm for layers consisting of spherical 3 µm particles. It is important that the overpressure be substantially higher than the applied pressure, so that the mobile phase is driven through the stationary phase. If this condition is not met, the pressurized membrane will not form a good seal on the plate surface [12].

3. Operating and development modes

Although OPLC can be started with a dry layer, as in classical TLC, the forced-flow technique also enables fully on-line separation in which the separation can be started on a stationary phase equilibrated with the mobile phase, as in HPLC. The following OPLC combinations [10] of the various off-line and on-line operating steps are feasible:

- fully off-line process: the principal steps, such as sample application, separation, and detection are performed as separate operations, similar to the conventional TLC or HPTLC method;
- off-line sample application and on-line separation and detection;
- on-line sample application and separation and off-line detection; and
- fully on-line process: the principal steps are performed as non-separate operations.

This operating mode is a form of HPLC that uses a 'planar column'. The versatility of the operating modes is summarized in Fig. 3.

OPLC may be performed in fully on-line mode where it is coupled to an HPLC detector, or in off-line mode where the plate is scanned in situ after the separation is finished. Considering the separation process between the off-line and on-line separation process, it can be stated that the largest difference is in the migration distance. In TLC, HPTLC and off-line OPLC at the end of the separation (the α front (the front of the first solvent in an eluent solvent mixture) of the mobile phase reaches the end of the layer) all compounds migrate a different distance, while using on-line LC methods, like

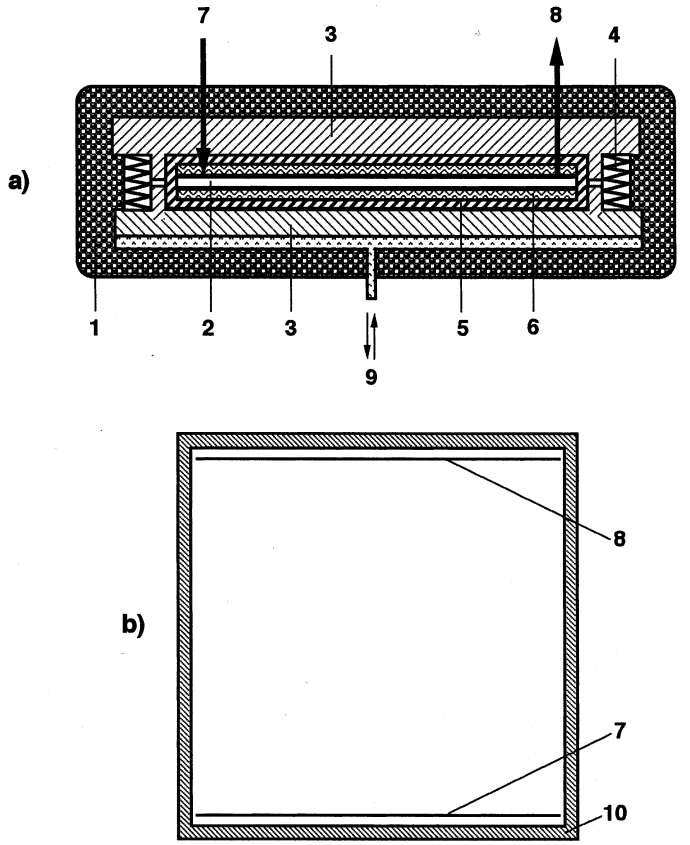

Fig. 2. (a) Schematic diagram of the commercially available cassette-type OPLC apparatus. 1 = support of the instrument, 2 = chromatoplate, 3 = inner part of the instrument, 4 = spring, 5 = cassette system for the chromatoplate between two teflon sheets, 6 = teflon sheets, 7 = mobile phase inlet, 8 = mobile phase outlet, 9 = hydraulic system. (b) Prepared plate for OPLC separation. 10 = polymer suspension for chromatoplate protection.

HPLC and on-line OPLC, all substances migrate the same separation distance [13], as shown in Fig. 4. With 20×20 cm HPTLC plates the fully on-line mode is limited to a single sample whereas the fully off-line mode can be used to separate several samples in a single run. In the fully off-line mode, the 'extra-column' effect is less important [9].

Comparison of the separation of fully on-line OPLC and HPLC reveals that the separation quality achieved by on-line OPLC is similar to that obtained by HPLC. Fig. 5 shows the separation of eight furocoumarin isomers. For all substances resolution and peak order were practically the same as in HPLC with the same mobile phase. The maximum cushion pressure of the applied chamber (25 bar) does not enable reduction of the separation time of 60 min by increasing the flow rate above 1.2 ml/min [14]. A substantially shorter analysis time can be obtained by use of the 50 bar overpressure now available with commercially available equipment. A comparison of applied HPLC, on-line, and off-line OPLC parameters for the furocoumarin isomers can be seen in Table 1.

In OPLC, the most frequent modes of development are linear one- and opposite-directional, as shown in Fig. 6a,b, respectively. In the latter the

Process / Steps	fully on-line	off-line & on-line	on-line & off-line	fully off-line
Sample application	continuous	separate	continuous	separate
Separation		continuous		separate
Detection			separate	separate

Fig. 3. Combinations of the various off-line and on-line operating steps.

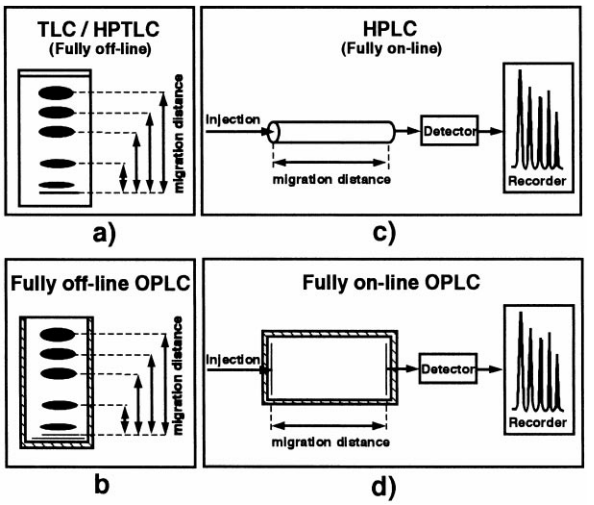

Fig. 4. Comparison of analytical LC methods, with reference to migration distances and operating modes. (a) TLC/HPTLC as fully off-line LC method. (b) Fully off-line OPLC. (c) HPLC as fully on-line LC method. (d) Fully on-line OPLC.

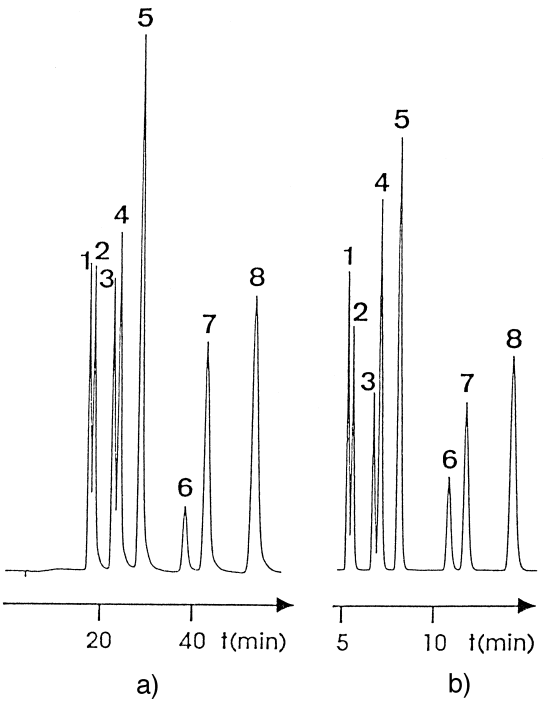

Fig. 5. Comparison of fully on-line OPLC and HPLC separations of furocoumarin isomers using the same stationary and mobile phases. (a) Fully on-line OPLC (flow rate 1.2 ml/min; counter pressure 23 bar). (b) HPLC separation (flow rate 2 ml/min pressure; pressure 250 bar).

mobile phase can be introduced in the middle of the plate, as illustrated in Fig. 6b. This mode is suitable only for samples containing a few components, as the separation path is limited to 9 cm [13]. Linear OPLC, however, requires a special chromatographic plate sealed along the edge, by impregnation. Using 20×20 cm chromatographic plates the maximum separation distance is 18 cm and 9 cm, respectively.

No preparation of the plate is necessary for off-line circular OPLC (Fig. 6c), where the mobile phase is delivered to the center of the plate, which results in a radial flow profile. If only a single sample is applied, it may be introduced (fully on-line, with 10 cm separation distance) into the

Table 1
Comparison of the parameters for the separation of furocoumarin isomers

	HPLC	Fully on-line OPLC	Fully off-line OPLC
Stationary phase			
– particle size (μm)	5	6	6
– volume (ml)	3.14	6.6	6.6
Separation distance (cm)	25	18	18
Mobile phase			
– equilibration time (min)	20	30	–
– flow rate (ml/min)	2	1.2	1.0
– max. counter pressure (bar)	250	50	50
Separation time (min)	15	60	20
Samples			
– number	1	1	18
– application	on-line	on-line	off-line
– amount/compound (μg)	0.2	0.4	0.04
– separation time/sample (min)	15	60	1.1
Detection	on-line	on-line	off-line
Total analysis time of one sample (calibration included) (min)	25	90	5

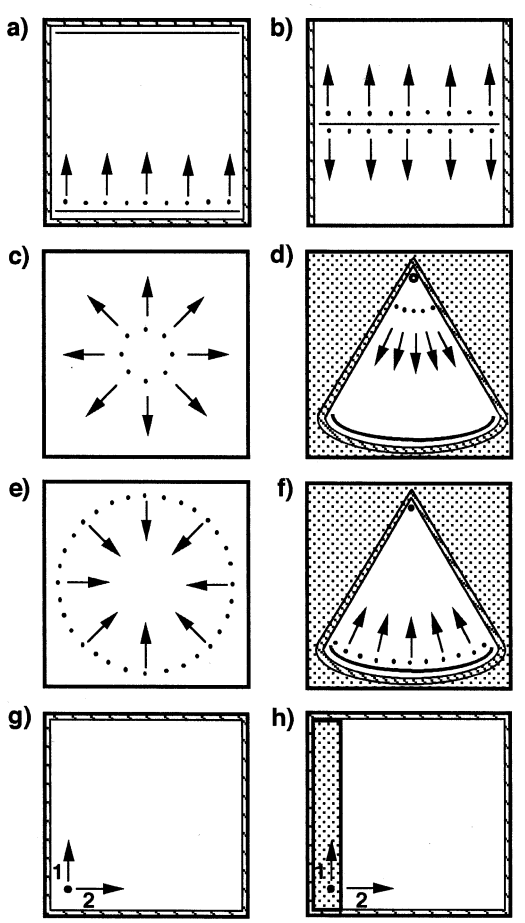

Fig. 6. Development modes of OPLC. (a) Linear one-directional development; 18 cm separation distance. (b) Linear opposite-directional development; 9 cm separation distance. (c) Circular development; 8 cm separation distance. (d) Circular development; 18 cm separation distance. (e) Anticircular development; 8 cm separation distance. (f) Anticircular development; 18 cm separation distance. (g) Two- or multi-dimensional development using monolayer stationary phase and 2×18 cm separation distance. (h) Two- or multi-dimensional development using bilayer stationary phases and 2×18 cm separation distance.

mobile phase stream, when the separated compounds will appear as a set of concentric circles. The advantage of this development, in which the mobile phase migrates radially from the center 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 higher resolution. This fact has been demonstrated by Kaiser in several articles, a summary of which is given in [10,13], respectively. The separating power of circular development is better exploited if the samples are spotted near the center. As the distance between the mobile phase inlet and sample application increases, the resolution begins to approach that of linear development. For on-line circular OPLC (Fig. 6d) a segment-shaped region must be isolated by removing the surrounding adsorbent [13], and its edges must be impregnated. An 18 cm separation distance thus be achieved.

However, the resolution is significantly higher in the upper R_F range using off-line anticircular separation (Fig. 6e) with a 9 cm separation distance, but the technique is rather difficult to perform because of the large perimeter of the mobile-phase inlet (ca. 60 cm for a 20×20 cm plate). Fully off-line and on-line 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 (Fig. 6f).

OPLC can also be performed in the bi-directional (Fig. 6g) mode [10]. The sample mixture is spotted near the corner of the chromatoplate and the first development is performed with a suitable mobile phase. After the first development the plate is removed from the equipment, the solvent is evaporated, and the plate is rotated through 90° and then replaced into the OPLC chamber. The second development is then performed with a second mobile phase of different solvent strength and selectivity. The separation power can be increased by performing the two developments on different stationary phases (bilayers), as shown in Fig. 6h.

4. Comparison of fully off-line and fully on-line OPLC separations

A comparison of the most important characteristics of off-line and on-line OPLC separations [14,15] is given in Table 2.

5. Special OPLC techniques

5.1. Multi-layer OPLC (ML-OPLC)

OPLC is suitable for the development of several chromatographic plates simultaneously if the plates are specially prepared [8]. Using this technique

several plates can be stacked face-up, on top of each other. In all chromatoplates – with the exception of the bottom plate – a small hole is located and the mobile phase is delivered to all plates simultaneously, through a channel formed by drilling to this small hole, by pressing the plates together (parallel connection). Fig. 7a shows linear ML-OPLC using three chromatoplates. The aluminum backing of the plate is sufficiently flexible to conform to the surface of the chromatoplates under conditions of OPLC. Needless to say, bi-directional linear OPLC can also be applied in the ML operating mode. Using the novel equipment (P-OPLC) for circular ML-OPLC (Fig. 7b) three HPTLC plates (72 samples/plate) can be used simultaneously for the separation of 216 samples. 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.

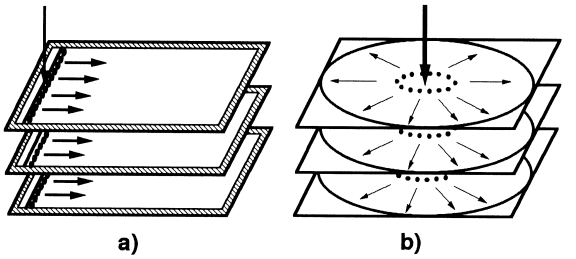

Fig. 7. Principle of multi-layer (ML) OPLC. (a) Linear one-directional development for the simultaneous separation of 54 samples; 18 cm separation distance. (b) Circular development for the simultaneous separation of 216 samples; 5 cm separation distance.

5.2. Long-distance OPLC (LD-OPLC)

LD-OPLC, developed by Botz et al. [16,17], is a multi-layer development technique with specially prepared flexible backing 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. Move-

Table 2
Comparison of fully off-line and fully on-line OPLC separations

	Fully off-line OPLC	Fully on-line OPLC
Separation	Development	Elution
Stationary phase	All commercially available	All commercially available
	Dry or wetted	Equilibrated
	Used once	Used several times
Mobile phase	Cut-off value not of importance	Cut-off value important in UV detection
Vapor phase	No, after prerun	No
Sample application	Static	Dynamic
	Prepurification not important	Prepurification necessary
	Solvent less important	Solvent important
	Several samples (18–216)	Only one sample
Development mode	Linear	Linear
	Bi-directional linear	
	Circular	
	Anticircular	
	Two-dimensional	
	Multiple	
Separation distance	≤54 cm	18 cm
Detection	Static	Dynamic
	Derivatization simple	Derivatization complicated
	UV/Vis (raw spectra)	UV/Vis (fine spectra)
	Fluorescence	Fluorescence
	–	NMR
	FTIR, in situ	–
	MS, in situ	MS
Evaluation	Densitogram	Chromatogram
	Repeatable	Unrepeatable

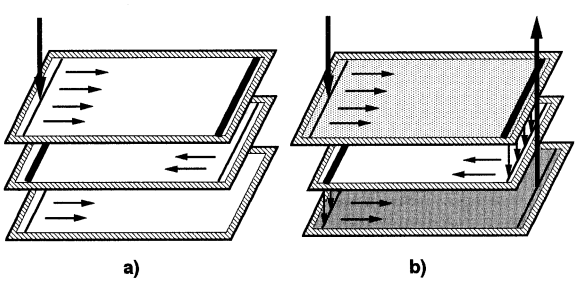

Fig. 8. Principle of long-distance (LD) OPLC. (a) Fully off-line OPLC using homolayer stationary phases. (b) Fully on-line OPLC using heterolayer stationary phases.

ment 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 a long running distance. A narrow slit (width about 0.1 mm) is cut at the end of the first (topmost) chromatographic plate to enable the mobile phase to travel to a second layer where the migration continues until the opposite end of the second (middle) layer, where solvent flow can be continued to the next subjacent (bottom) chromatographic plate, or the eluent is led away if migration is complete. Clearly, on this basis a 54 cm separation distance can be achieved by connecting three plates together. In the arrangement presented (Fig. 8a) 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 enables ready passage of the mobile phase and individual samples without any mixing. The cushion of the OPLC instrument is applied to the uppermost layer only, and each plate presses on to the sorbent layer below. As a consequence, glass-backed plates can be used in the lowest position only. The illustrated fully off-line separation is complete when the α front 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 [10]. The eluate can, furthermore, be led from the lower plate, similarly to the way it was led in Fig. 8b. This gives the possibility of on-line detection. For this fully on-line operating mode all layers placed between the highest and lowest plates must have 1 cm cut from the length of the plate, to leave a space for mobile phase outlet.

5.3. Isolation using analytical plates

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 a single HPTLC plate. This can be increased threefold by use of three 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 30 mg on a single chromatographic plate. Linear on-line OPLC is preferable if the structures of the compounds to be separated are similar [10]. The circular off-line technique can be used if the separation problem is in the lower R_F range, as demonstrated in Fig. 9, where the off-line micropreparative separation of a ginseng extract is illustrated.

A special clean-up effect, sample application and reconcentration, can be achieved simultaneously using the configuration in which the upper plate serves for clean-up. These steps can be freely chosen combinations of different off-line and on-line steps. The potential of linear on-line OPLC on 20×20 cm preparative plates as a preparative method is considerable – separation of six to eight compounds in amounts up to 300 mg. Because the distribution and the average particle size of precoated preparative plates is too large, not all the

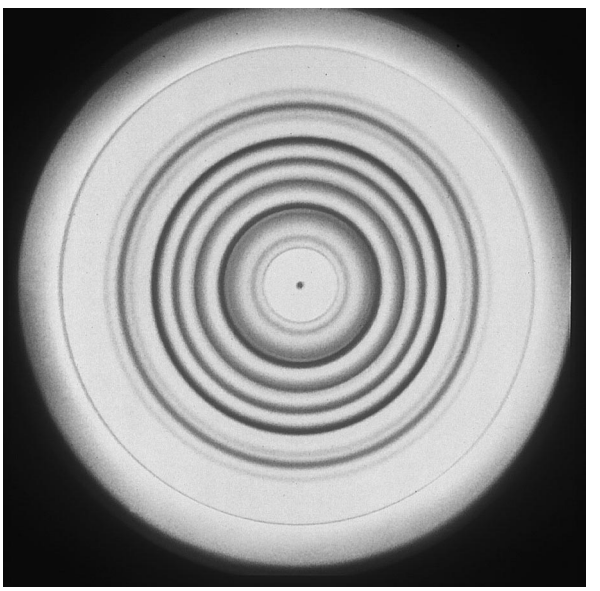

Fig. 9. Isolation of ginsenosides on an analytical HPTLC plate with circular off-line OPLC.

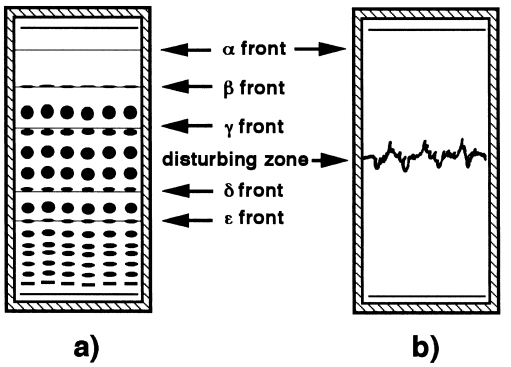

Fig. 10. Elimination of typical problems by use of OPLC. (a) 'Multi-front effect', a consequence of demixing of the multi-component mobile phase. (b) 'Disturbing zone', the extent of this phenomenon depends on the interrelationship between gas physically bound to the surface of the sorbent and gas molecules dissolved in the mobile phase.

advantages of this preparative method can yet be realized.

6. Elimination of typical problems arising during the use of OPLC

Solvent demixing [12] can occur in any mode of planar chromatography when the mobile phase consists of two or more solvents of different polarity. For multi-component mobile phases with n constituents, n fronts can occur (see Fig. 10a). This phenomenon occurs only if the separation starts with a non-equilibrated layer, as with off-line separations. Compounds that migrate with a front cannot be separated from each other. Densitometrically a very sharp peak is obtained when scanning such a substance, and it can be used to the analyst's advantage; this can be automatically subtracted from the compound peak by scanning the front in a lane where sample has not been spotted. Note that this 'multi-front effect' also has a positive effect in preparative separations, because compounds which migrate with a front can be eluted in a very small amount of mobile phase. Demixing does not occur with single-solvent mobile phases, and it is usually not a problem when working with reversed phase plates. It is usually possible to find a set of experimental conditions such that demixing is not a problem.

The 'disturbing zone' is another curse of a secondary front in OPLC. If the 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 [12]. This effect appears during the chromatographic process as a zigzag 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 Fig. 10b. 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. The 'disturbing zone' can be prevented by performing a prerun with any compo-

Table 3
Connection between development mode, separation distance and number of samples using a fully off-line operating mode

Development mode		Separation distance (cm)	Number of samples
Linear	one-directional	18	18
	two-directional	9	36
	one-directional, ML	18	54
	two-directional, ML	9	108
	one-directional, LD	54	18
Circular		9	1–72
	ML	9	216
	specially prepared	18	6
	specially prepared, ML	18	18
Anticircular		9	1–72
	specially prepared	18	6
	specially prepared, ML	18	18
Bi-directional (multi-dimensional)		2×18	1
	ML	2×18	3

nent 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.

Last but not least, proper preparation of the chromatoplate (efficient impregnation with the polymer suspension) is important, as are the selection of appropriate inlet pressure [12] and ensuring that the overpressure is substantially higher than the pressure used to drive the mobile phase through the stationary phase. If these conditions are not met the efficiency of the OPLC technique will be reduced.

7. Correlation between OPLC and HPLC retention data

Because OPLC may be used as a non-equilibrated (off-line) or equilibrated (on-line) planar column system and the fronts of the multi-component mobile phase can be seen, it can be applied as a pilot method for the various pressurized preparative LC techniques, like FC, LPLC, MPLC and preparative HPLC. Depending on the results of the analytical OPLC separation, four possibilities exist for mobile phase transfer to MPLC [18].

Using silica as the stationary phase, the generally useful method is to equilibrate the dry-filled column (TLC quality with average particle size 15 μm) 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 and the substances are distributed over the whole R_F range. Transfer of the optimized TLC mobile phase via OPLC to

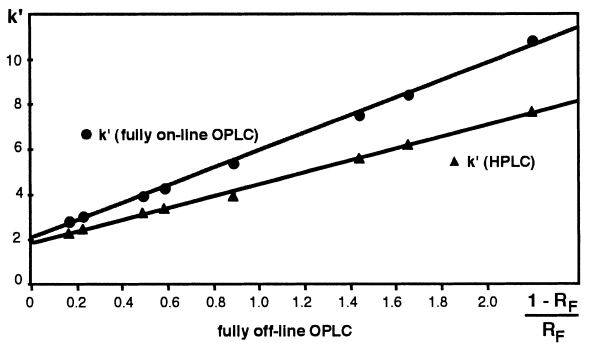

Fig. 11. Correlations between retention data from fully off-line OPLC and fully on-line OPLC and HPLC.

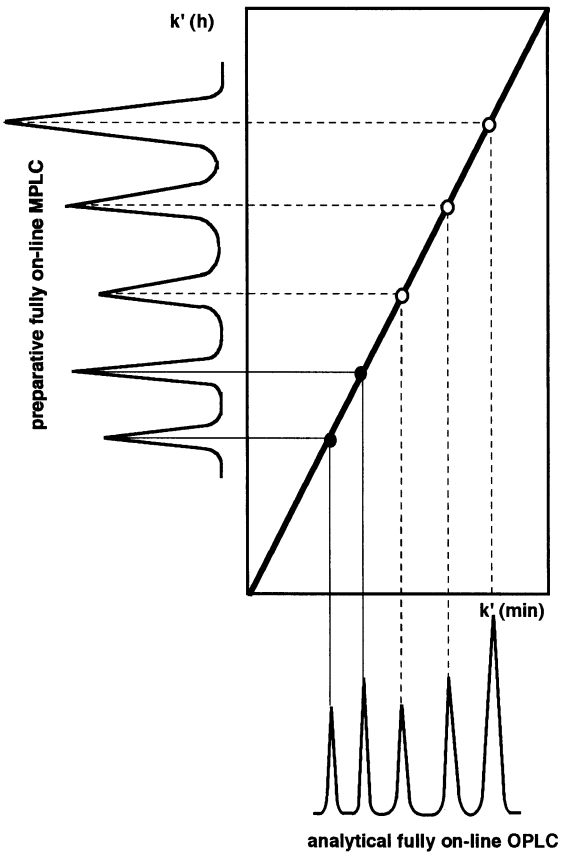

Fig. 12. Prediction of the k' values of a preparative MPLC separation from the retention data from analytical fully on-line OPLC.

MPLC is demonstrated by the separation of furocoumarin isomers from the roots of *Heracleum sphondylium*, the ginsenosides from *Panax ginseng* C.A. Meyer and the anthraquinone aglycones from *Rhamnus frangula* [18]. The correlation of retention data from fully off-line OPLC with those from fully on-line OPLC and HPLC [15] are given in Fig. 11. Because of these linear relationships the separation times in on-line OPLC or HPLC can be predicted for all compounds (after the elution of the first three peaks) from the off-line OPLC R_F values. Prediction of preparative MPLC is always possible if retention data from the analytical on-line OPLC are known [18]. Analytical retention data from the first two peaks (filled circles in Fig. 12) and the zero point enables the calculation of the retention times of th missing compounds in the preparative separation (open circles in Fig. 12). Clearly the resolution can also be predicted in this manner.

8. Advantages of OPLC

The advantages of the different OPLC methods [10] and techniques [19] can be summarized as follows:

- OPLC serves as a 'bridge' between planar and column liquid chromatography, since it can be used either as a fully off-line (similar to HPTLC) or a fully on-line (similar to HPLC) technique, or applied as a free combination of the three basic steps: sample application, separation and detection.
- OPLC increases the separation power and the number of samples analyzed, due to

  the forced flow,
  the selection of the appropriate development mode,
  the choice of a suitable separation distance,
  the number (≤ 3) and quality (homo or hetero) of chromatoplates used in parallel (ML technique) or serial connection (LD technique), and
  the second, or multi-dimension.

Some of these effects using a fully off-line operating mode are summarized in Table 3.

- OPLC enables the ML technique to be used for micropreparative purposes also on identical plates as well as the application of different stationary phases and LD techniques for analysis or isolation purposes.
- OPLC enables mobile phase transfer between TLC and the various column liquid chromatographic techniques. Mobile phases optimized in unsaturated analytical TLC can be transferred after a suitable prerun to analytical OPLC, and from this to analytical HPLC. The direct transfer of the mobile phase from OPLC to different preparative column chromatographic methods (FC, LPLC, MPLC, semi-preparative HPLC) is also possible.
- OPLC enables the prediction of elution time and the quality of separation of compounds to be isolated using FC, LPLC, MPLC, or semipreparative HPLC techniques from data from analytical OPLC separations.
- In OPLC the HPTLC plate is used only once and may be discarded after use. This makes it particularly suitable for analyzing complex biological samples that would require clean-up before HPLC.

9. Future of OPLC

The above overview of the present status of OPLC covers a special range of analytical methods, the characteristics of which lie between conventional off-line HPTLC and modern, on-line HPLC [19,20]. OPLC, especially with the selection of operating and development mode and the selection of parallel or serial connection of plates, covers a special range of modern instrumental separations. It does not compete with HPTLC or HPLC. Instead, the three approaches are complementary and together they make for successful and rapid separation. In our opinion OPLC has and will probably always have a role in the analysis of pharmaceuticals [21–23], drugs [24,25], foods [26,27] and toxicological samples [28,29], as well as in environmental [30] analysis.

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