Ph.D. Thesis

Overpressured layer chromatography (OPLC) with special emphasis on the separations in the sorbent layer segmented by flowing eluent wall

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Overpressured layer chromatography (OPLC) with special emphasis on the separations in the sorbent layer segmented by flowing eluent wall

1. The place and necessity of OPLC among liquid chromatographic techniques

Conventional planar and nonplanar as well as thin- and thick-layer liquid chromatographic techniques require few instruments and are rather simple. Among the planar layer liquid chromatographic techniques, paper chromatography (PC) and its various versions, developed in the 1940s by Martin et al., have to be mentioned first (1). Thin-layer chromatography (TLC), discovered by Ismailov and Shraiber (2) as well as Békésy (3), improved by Kirchner et al. (4), and standardized and spread by Stahl et al. (5,6), contributed to the isolation and analysis of many natural and synthetic substances. Today, versions of this classical chromatographic technique are indispensable in different fields of scientific research and practice.

The combination of the flame ionization detector (FID) with TLC (TLC/FID as a nonplanar layer chromatographic technique) gives quantitative results without the need to use color reagents. In this system the thin sorbent layer is, e.g., on a glass rod (open or turned out column) (7).

Column and layer liquid chromatographic techniques - as supplementary techniques due to their arrangements - have always been characteristically developed in constant mutual interaction. Hence it is not surprising that the intensive development of high-performance column liquid chromatography (HPLC) entailed the need for the fundamental renewal of the most popular planar layer liquid chromatographic technique, TLC. In light of this it can also be understood that the latest efforts aimed at the further development of layer liquid chromatography are characterized by the desire to introduce sophisticated instrumental techniques similar to HPLC (8-10).
Attempts to develop an ultramicro (UM) chamber were first made in the early 1970s (11). In this simple chamber the chromatoplate is covered by a glass plate so that the end of the cover plate is not immersed in the solvent. This chamber is well suited for modeling classical column chromatographic (CC) separation.

Important new instruments were developed after the UM chamber, aimed at increasing the efficiency of TLC through improvement of the separation mechanism. Programmed multiple-development TLC as elaborated by Perry (12) combines the techniques of continuous multiple development and evaporation. Recently this technique was improved by Burger (13). In this system the chromatoplate is developed several times in the same direction with various eluents of decreasing elution power. Between developments the chromatoplate is dried by vacuum. This method is termed automated multiple development (AMD) (14). High-performance TLC (HPTLC) is based on the use of chromatoplates coated with fine particles of narrow particle size distribution sorbent and is carried out with sophisticated instrumentation (15,16).

Modern methods of column liquid chromatography employ constant flow rates (8-10), although this has not been the case in TLC and HPTLC. The greatly increased

*Bold number refers to own result*
developing time on a fine-particle-size sorbent layer (HPTLC chromatoplate) made it necessary to employ forced flow, which is also exploited in centrifugal layer chromatography (CLC) (17) [a new term is rotation planar chromatography (RPC) (18)], and in high-speed TLC (HSTLC) using electroosmosis to force the eluent (19).

However, the first successful step to a real planar version of HPLC was the development of a pressurized ultramicro chamber, the basic instrument of overpressured layer chromatography (OPLC) (20-22), using a pump system for admission of the eluent. The infusion and transfusion (23) off-line and on-line operating modes in OPLC and their combination (24), as well as the parallel (25) and serial coupled (26) multi-layer systems are basic technical versions of OPLC. The development of flowing eluent wall (FEW) procedure seems to be a real solution of fully on-line multi-channel liquid chromatographic technique using non-segmented sorbent bed (27, 28). Automated OPLC 50 system (29) provides a user friendly, automatique, accurate and sensitive solution of the original technique (20-22).

Figure 1 illustrates the place of OPLC techniques among the basic column and layer liquid chromatographic techniques classified according to the mode of transport of the mobile phase and the shape of the sorbent bed.

3 The theoretical basis of OPLC

3.1 The rule of mobile phase movement and factors influencing retention

3.1.1 Formation and migration of fronts in fully off-line system

It is well-known in conventional layer chromatography (TLC, HPTLC, preparative layer chromatography (PLC)), that the eluent migrates by means of capillary forces and a quadratic equation exits (30-32).

\[ z_f^2 = k \cdot t \]

where \( z_f \) is the distance of visible \( \alpha \) front, \( t \) is the time of development, and \( k \) is the velocity constant.

In the case of OPLC the eluent can be forced through (or into in case of infusion operation) the sorbent bed by means of a pump system using a chosen flow rate (20).
Feeding the eluent by constant velocity, the speed of the front depends on the cross-sectional area of the sorbent layer in the direction of the development. Only linear developments are able to result in constant linear velocity; other geometric shapes of sorbent layers (circular, triangular) are not.

Accordingly, the basic flow rule of linear transfusion OPLC is (33, 34)

\[ z_f = u \cdot t \]

**Bold number refers to own result**
where \( z_f \) is the migration distance of the eluent front, \( u \) is the linear migration velocity of the eluent front, and \( t \) is the time of the development.

This means that in linear OPLC the velocity is constant along the plate, in contrast to the circular version of OPLC, where the velocities of fronts and components decrease along the radius during the developing time.

Figure 2a illustrates the basic differences among the conditions of eluent flow in conventional layer chromatography, one- and two-directional linear and circular transfusion OPLC applying constant flow rate (35, 36).

As it can be seen in Figure 2b, the theoretical line of linear transfusion OPLC development intersects the curve of conventional development, and the linear velocity is initially higher than the one for OPLC. A starting rapid eluent flash (e.g., the use of pressurized buffer space) results in high velocity, and curve 3 is continuously higher than curve 1. The automated OPLC 50 system automatically manages this period dividing the process into two parts (line 4). The initial rapid period, having higher velocity, ensures the formation of a straight front by quick wetting of the sorbent layer at trough area. A period of lower velocity of separation follows this high-velocity step. At a distance (position 5) the velocity becomes constant, and samples should be applied up to this limit. In the case of infusion OPLC process, the speed of alpha front decreases continuously, whereas the mobile phase inlet pressure increases with continuously increasing speed during development (23).

3.1.2 Formation and migration of front of total wetness in fully off-line system

If a dry porous sorbent bed made of irregular sorbent is used at the beginning of development, two zones can be found with significant differences in their refractive indexes, even if single eluents and conventional (32, 37) or forced-flow layer chromatographic techniques are used (38, 39, 40).

In the case of classical, fully off-line transfusion OPLC (transfusion operation: the sorbent layer is "open-ended", the opposite side of the eluent inlet is open, through out an eluent outlet tube), in the zone under the \( \alpha \) front \( (F_{\alpha}) \), the space between the sorbent...
particles and within the pores is filled partially with air and eluent. This is called the partially wetted zone ($z_{pw}$), which sometimes disturbs the separation, in this narrow range ($38, 41$) (Figure 3). The next zone toward the eluent inlet point is a totally wetted one ($z_{tw}$), which is completely filled by the eluent. The border between these zones is the front of total wetness ($F_{tw}$) (Nyiredy et al. termed “disturbing front” (39)), which is not straight in most cases but a sharp zigzag line arises due to the inhomogeneity of external and internal pore diameters of the sorbent bed.

In fully off-line transfusion OPLC is the $F_{tw}$ and the components migrate proportionally with $F_\alpha$ at a constant flow rate ($38, 39, 40$). $F_{tw}$ changes from a straight line to a zigzag one during the separation, and its bandwidth increases with migration distance. This effect is greater on a TLC plate than on an HPTLC plate. The waviness of

**Figure 3** Migration of the solvent fronts and substances during continuous development using transfusion OPLC technique (38).
Chromatographic conditions: (Chrompres 25); silica gel 60 (Merck); isooctane-THF (100:7.5); external pressure, 2.0 MPa. $L$, migration distance; $S$, start point; $I$, eluent inlet point; $O$, eluent outlet point; $P_E$, inlet pressure. 1, $\alpha$ front; 2, front of total wetness ($F_{tw}$); 3, $\beta$ front ($F_\beta$); 4, inlet pressure ($P_E$); 5, curve of eluent volume at outlet ($V_E$); 6-10, substances separated (6, blue dye, eluting in $F_\beta$ 7, perylene; 8, yellow dye; 9, pink dye; 10, red dye). Stages of continuous development: I, classical, fully off-line OPLC; II, leaving of partially wetted zone; III, leaving of secondary fronts; IV, equilibration.
$F_{tw}$ can be dramatically reduced applying a higher external pressure on the layer surface (42). In contrast to the transfusion process, infusion yields a continuously decreasing waviness of front of $F_{tw}$ as well as sample band shape of that area during development. The air originally contained the sorbent is continuously compressed, helping to fill the pores of particles. Figure 4 shows the differences among transfusion,
infusion and infusion-transfusion operation during the run. Contrary to the transfusion process, the infusion-transfusion process yields a quick stabilization concerning the eluent/sorbent ratio resulting better conditions for on-line separation-detection process (23, 43).

Nyiredy et al. (40) defined a critical pressure, which can be related to $F_{tw}$. The $R_f$ value of $F_{tw}$ ($R_{tw}$) may vary with the conditions of development.

Velayundhan et al. (40) found that $R_{tw}$ linearly increases with the flow rate but $F_\alpha$ shows slight nonlinearity at higher flow rates. This phenomenon is independent of the viscosity of applied eluents (methanol, ethanol, and heptane). The pressure drop linearly increases with the migration distance and the time of development. It depends on the viscosity of the eluent, the particle size of the sorbent layer. Within experimental error their incompressible model is in agreement with experiments, and the velocities of the fronts are:

$$U_{F_\alpha} = (1 + a)U_{F_{tw}} \quad \text{and} \quad a = \varepsilon_p / \varepsilon_i$$

where $\varepsilon_i$ is the interstitial and $\varepsilon_p$ is the intraparticulate porosity per total volume of bed.

If the sorbent layer is not wettable by the eluent, e.g., in the case of reversed-phase

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{wetting_profiles.png}
\caption{Wetting profiles of transfusion (T) an infusion (I) off-line OPLC (23). 1, print of eluent inlet trough; 2, front of total wetness; 3, alpha front.}
\end{figure}

**Bold number refers to own result**
sorbent applied in water elution, $F_{tw}$ migrates together with $F_\alpha$ (40).

Figure 5 clearly shows the differences between transfusion and infusion operations concerning the eluent filling level of the sorbent layer (23).

In transfusion off-line operation the sorbent/eluent ratio is not constant along the plate due to the partially filled zone. Owing to this fact the front distance is always longer than the real one measured at totally filled conditions. It can be concluded that infusion operation mode needs more eluent (about 10%) (23).

Using diagonal sample application and single eluent, $R_F$ values were practically independent of spotting location, and their values were higher on HPTLC layers than on TLC layers (21).

3.1.3 Formation and migration of secondary fronts

It is a well-known fact in classical TLC that the eluent components sorbed strongly by the sorbent sites can cause secondary fronts ($F_\beta$, $F_\gamma$, ...) (Niederwieser and Brenner (44)). This effect can be found during adsorption as well as in reversed-phase development when the eluent consists of solvents of different strength.

The effect of this chromatographic solvent demixing is stronger in fully off-line OPLC systems (38), owing to the total elimination of vapor space, than in chambers with small vapor spaces, e.g., in sandwich chambers. These secondary fronts (that are independent of $F_{tw}$) divide the sorbent layer into zones of different eluting strength, within which the solvent strength and polarity are practically the same, while at the fronts themselves there is a sudden increase in eluent strength that gives rise to "polarity steps". This phenomenon takes place in HPLC (45) and in fully on-line OPLC as well, but only after eluent changes during equilibration, when the apparatus is not used for separation (38, 41) (See Fig.3).

Eluent strength was correlated with $R_f, \beta$ using fully off-line OPLC, silica gel 60 and different apolar and polar eluent mixtures. (The eluent strength ($\varepsilon$) of a given eluent
mixture can be calculated according to Snyder and Glajch (46)). The mixtures of hexane and ethyl acetate or tetrahydrofurane or acetone show linear relationships between $R_f, \beta$ and $\epsilon$. The mixtures of ethyl acetate and carbon tetrachloride, or benzene, or methylene chloride fail to show this type of correlation (41).

The eluent strength of $\beta$ zone is regarded to be similar to the calculated one. If the secondary front collects analyzable components from the preceding zone ($\alpha$ zone), shorter development or higher sample origin is needed. When sample components are not sensitive and $\alpha$ zone can elute component collected by secondary front, double development with same eluent can be used. If the phenomena can not be overcome new eluent should be used. If the polar constituent of the used eluent is replaced by a weaker one of the same volumetric ratio, higher $R_f, \beta$ and lower $\epsilon$ value of $\beta$ zone arise. Replacing of the apolar constituent of this new eluent to stronger one results in higher $R_f, \beta$ and higher $\epsilon$ value of $\beta$ zone. At a given eluent composition and sorbent, the $R_f, \beta$

**Figure 6** Effect of eluent composition on $R_M$ values in fully off-line OPLC (48). Eternal pressure on membrane, 1.2 MPa; temperature, 30 °C; TLC silica gel (activated at 125 °C for 30 min); eluent, benzene-ethyl acetate mixture; linear velocity, 1.2 cm/min; development distance, 165 mm; samples, dye mixture; 1, violet; 2, yellow; 3, red; 4, $\beta$ front on silica gel 60; 5, pink; 6, $\beta$ front on silica gel 40; 7, $\beta$ front on silica gel 100.
value is constant, independent of migration distance and velocity of eluent used (35, 38, 47).

The $R_f$ of a secondary front depends on the eluent composition. It was found that a plot of $R_M$ versus the logarithm of the mole fraction of polar constituent used in the mixture did not show linear relationship, unlike the compounds' migration in the $\beta$ zone. $R_{f,\beta}$ increases with increasing concentration of polar modifier as well as with decreasing specific surface areas of the sorbent (48) (Figure 6).

Similar results were found by Wawrzynowicz and Soczewinski (49) in case of a sandwich chamber and binary eluents.

Markowski et al. (50) have applied sandwich TLC for the evaluation of adsorption isotherms, comparing this method to the breakthrough and static methods. All the three methods have given similar results.

A vapour pretreatment of the sorbent layer was applied by Ojanperä et al. prior to OPLC development for the elimination of secondary front effect (51).

3.1.4 Retention transfer among TLC, off-line and on-line OPLC and HPLC

The elimination of vapour phase above the sorbent layer in OPLC may cause disturbances in the retention transfer from TLC to OPLC.

Retention data obtained in fully off-line OPLC can be converted to on-line separation/detection conditions by the help of equation found Schlitt and Geiss (52):

$$k = \frac{1}{R_f} - 1$$

where $k$ is the capacity factor of a given component in the on-line system.

Strong correlation was found on silica gel layers among fully off-line, partially off-line (off-line sample application, on-line separation/detection) and fully on-line OPLC, even if eluents were used with more components (41). The slope of the line is not 1 due
to the difference of sorbent bed conditions. If the $\beta$ front collects some components, the concept of $R_M$ additivity can be used to convert these data into those of the fully on-line system:

$$R_{M,i}^W = R_{M,\beta} + R_{M,i}$$

where $R_{M,i}^W$ is the $R_M$ value in the wet system, $R_{M,\beta}$ is the $R_M$ value of the $\beta$ front, and $R_{M,i}$ is the $R_M$ value of the given collected components in the $\alpha$ zone.

If the number of silanols in silica gel is reduced by a polar silane reagent - for example, 3-glycidyloxypropyltrimethoxysilane, the resulting diol-modified layer is less sensitive to relative humidity, and $R_f$ values are generally higher on it than on bare silica layers. Thus the modified layer is suitable for the separation of nonpolar and polar compounds using simple, less polar eluents. The correlation between the retention data of fully off-line and fully on-line OPLC is stronger than it is on silica layers (48)(Figure 7).

Reversed-phase ion-pair chromatography can be optimized by fully off-line OPLC according to Szepesi et al. (53). Good agreement was found in selectivity between HPLC and OPLC ion-pair systems using the same eluent composition by Gazdag et al.,

**Figure 7** Rapid (a) fully off-line, (b) fully on-line OPLC separation, and (c) comparison of retention data (48).

Conventional OPLC chamber, external pressure on membrane, 2.8 MPa; temperature, 23°C; diol-modified HPTLC silica gel 60; eluent, n-hexane; flow rate, 2.5 cm$^3$/min; detection, absorbance at 254 nm.

Sample volume injected and streaked 3 µl, dissolved in carbon tetrachloride: 1, carbon tetrachloride; 2, toluene; 3,acenaphthene; 4, phenanthrene; 5, pyrene; 6, chrysene; 7, benzopyrene; 8, butter yellow; 9, fat red.
and this made the modeling of HPLC ion-pair systems by fully off-line OPLC possible (54).

Selectivity of the mobile phase for coumarins was similar in TLC, off-line OPLC and HPLC as found Vuorela et al. (55). The change of eluent strength had the same effect on retention using TLC and OPLC as non equilibrated systems.

The retention transfer is influenced by the eluent/sorbent ratio as well as the sorbent activity. Owing to eluent/sorbent ratio is not constant along the plate due to the partially filled zone in transfusion off-line operation, front distance is always longer than the real one measured at totally filled conditions. In contrast to that in infusion operation the eluent/sorbent ratio is practically constant along the sorbent layer and the value is equal to the ratio of the totally wetted zone of transfusion operation. Because of the off-line processes starts with dry sorbent bed, during the equilibration the sorbent bed activity may change resulting an altered retention for on-line OPLC (35).

3.2 Efficiency characteristics of OPLC and theirs influencing factors

3.2.1 Theoretical plate height

In conventional layer chromatography the theoretical plate height (HETP) can be calculated according to Guiochon and Siouffi (56), and it is also applicable to off-line OPLC systems (21). HETP \( H \) is as follows:

\[
H = \frac{\sigma^2}{(L_f - s_o)R_f}
\]

where \( \sigma \) is the spot variance, \( L_f \) is the front distance, \( s_o \) is the distance between the spotting location and the eluent inlet trough, and \( R_f \) is the retention factor.

Owing to the effect of focusing, an initial (starting) spot width may be defined that is different from the spot width deposited. The initial spot variance \( \sigma_{oi}^* \) of a given compound (i) is

\[
\sigma_{oi}^* = \sigma_o^* (1 - R_{Efi})R_{Sfi}
\]

where \( \sigma_o^* \) is the spot variance of solvent deposited and \( R_{Sfi} \) and \( R_{Efi} \) are retention factors in the solvent and eluent, respectively (48).
If the bandwidth of the spot or band deposited is very narrow in off-line OPLC, HETP is practically constant along the sorbent layer and independent of the front distance \((21, 42)\). Figure 8 illustrates clearly the basic difference between TLC, HPTLC, and off-line OPLC with respect to efficiency. Because of the significant reduction of waviness of \(F_{nv}\) and also the component band sizes of that area the efficiency is increased during infusion off-line OPLC development \((23)\).

In plotting data for HETP versus the migration distance of different compounds \(L_i\), a gradually decreasing curve was obtained. This curve was linearized by plotting

\[
\text{HETP} = \frac{1}{L_i} 
\]

The linear relationship between the HETP and the inverse migration distance of a component \(L_i\) can assume the following HETP calculation \((48)\):

**Figure 8** Variation of theoretical plate heights (H) of PTH-valine along the plate with a conventional normal unsaturated chamber and automated OPLC system \((42)\). 1, HPTLC silica gel, normal unsaturated chamber; 2, TLC silica gel, normal unsaturated chamber; 3, TLC silica gel, OPLC; 4, HPTLC silica gel, OPLC, 5 MPa; 5, Raman silica gel, OPLC, 5 MPa; Sample application: TLC silica gel (thickness, 0.220 mm), 0.2 µl; HPTLC silica gel (thickness, 0.160 mm), 0.1 µl; Raman silica gel (thickness, 0.095 mm), 0.05 µl; \(L_i\), front distance.
\[ H_i = \left( \frac{\sigma_{io}^*}{L_i} \right)^2 + H_\infty \]

where \( \sigma_{io}^* \) is the initial spot variance and \( H_\infty \) is the HETP at the point of intersection. It was assumed that \( H_\infty = hdp \), where \( h \) is the reduced plate height and \( dp \) is the particle diameter according to Knox (57).

In OPLC the HETP depends on the characteristics of the plate used and decreases in the following order: preparative layer, TLC, HPTLC and RAMAN plate (38, 41, 42).

Hauck and Jost have also found that HETP depends on the \( R_f \) and front distances (58). The thickness of sorbent layer influences HETP slightly (58). In off-line OPLC, HETP may vary with the linear velocity (58, 59), similarly to HPLC.

HETP depends in OPLC on the combination of the off-line and on-line operating steps applied (41, 48). Figure 9 shows HETP (\( H \)) versus linear velocity (\( u \)) using different operating modes of transfusion OPLC. The curves are very similar, but the values of HETP are different. The fully off-line OPLC produces the lowest and the fully

**Figure 9** Relationship between theoretical plate height (\( H \)) and eluent front velocity (\( u \)) for different modes of OPLC (48).

Chrompres 25; external pressure on membrane, 2.8 MPa; HPTLC silica gel 60; dichloromethane-ethyl acetate (9:1); PTH-methionine; bandwidth deposited and trough width, 0.68 mm; migration distance, 175-180 mm for off-line detection and 180 mm for on-line detection. 1, Fully on-line OPLC; 2, on-line sample application/separation and off-line detection; 3, of-line sample application and on-line separation/detection; 4, fully off-line OPLC.
on-line OPLC the highest HETP values. Between them are the two curves of partial off-
line (or partial on-line) OPLC. The differences among these systems originate from
"extra-column" band broadening, which does not occur in the fully off-line system.

Roeraade and Flodberg found, that the quality of the packing can also influence
HETP values, and values can be decreased by compression of the sorbent layer up to a
limited external pressure (60).

The elevation of external pressure decreases the HETP value under off-line OPLC.

![Figure 10](image)

**Figure 10** Effect of linear velocity (u) on theoretical plate height (H) using different
external pressures and fully off-line transfusion OPLC (42). Eluent, dichloromethane-ethyl acetate (98:2); HPTLC silica gel; PTH-valine, 0.4 mg/ml; 5 µl/10 mm band;
1, 1 MPa; 2, 2.5 MPa; 3, 5 MPa.

But it doesn't occur during on-line development (61). (The results measured by
Flodberg and Roeraade were similar if 3 µm spherical sorbent layer was used (62)).
Figure 10 shows the effect of linear velocity on HETP at three different external
pressures using transfusion off-line operation and fine particle silica. The high external
pressure significantly increases the efficiency. Furthermore, the optimum range of linear
velocity becomes broader (42).

3.2.2 *Theoretical plate number*

*Bold number refers to own result*
The theoretical plate number \((N)\) can be calculated by the subsequent equation in OPLC as well

\[ N = \frac{L_f}{H} \]

where \(L_f\) is the distance between start and front and \(H\) is the average theoretical plate height.

If the bandwidth of the deposited spot is very narrow in OPLC, HETP is practically constant along the plate (21). It means that the theoretical plate number increases linearly with development distance contrary to TLC/HPTLC.

The theoretical plate number (as well as the spot and/or peak capacity) can be increased by multi-layer OPLC using layer connection in series (called “long distance” OPLC according to Botz et al.) and 70·10³ theoretical plate number was achieved using butter yellow and 70 cm long development (63).

### 3.2.3 Spot and peak capacity

The spot capacity of conventional layer chromatography is limited, as found Guiochon and Siouffi, and about 20 spots can be resolved with a resolution of unity (64).

In the case of thin column (and also in fully off-line OPLC), the maximum value of spot capacity \((n_M)\) can be calculated according to Guiochon at al. (65):

\[ n_M = \frac{1}{2} \sqrt{\frac{L}{H}} \]

where \(L\) is the distance of development and \(H\) is the average HETP value of compounds at given conditions.

Peak capacity \((n)\) of a column in HPLC is given by the well-known equation (64-66), and it is also applicable for the on-line separation/detection OPLC systems (24):

\[ n = 1 + \sqrt[2]{\frac{N}{4}} \ln(1 + k) \]

where \(N\) is the theoretical plate number of the sorbent bed at given conditions and \(k\) is the capacity factor of the most retained compound eluted.

**Bold number refers to own result**
Owing to the openable sorbent bed after an on-line separation/detection, compounds separated but remaining on the layer can also be detected in situ by densitometry. In this combined on-line and off-line OPLC system, higher spot and/or peak capacity can be observed at given bed length than by single on-line or single off-line OPLC separation, because the peak and spot capacities are additive (24) (Figure 11).

3.2.4 Resolution

The resolution of a neighboring pair of spots can be described by the next equation:

$$R_S = \frac{1}{4} \left( \frac{K_1}{K_2} - 1 \right) \sqrt{R_f N (1 - R_f)}$$

where $K_1, K_2$ are distribution coefficients of two substances, $R_f$ is the average retention factor of pairs and the $N$ is the theoretical plate height.
The plot of resolution vs. $R_f$ shows the differences between TLC/HPTLC and OPLC. Figure 12 illustrates that in TLC the optimum range of resolution is $R_f 0.3-0.4$ and in OPLC where the $R_s$ range is $R_f 0.3-0.65$ (67).

Three factors were studied by Hauck and Jost regarding the resolution using fully off-line OPLC (58). Use of the optimal linear flow velocity in relation to HETP produces the highest resolution. The relationship between resolution and distance of development is approximately linear, and the resolution increases with increasing front distance. The layer thickness shows an optimum value (80-160 µm) in terms of resolution.

**Figure 12** Relationship between $R_s$ (Y) and $R_f$ (average $R_f$ value; X) for reversed phase chromatography of aldehyde DNPH derivatives (67) 1, off-line OPLC; 2, TLC

4 The basic elements and instruments of OPLC

4.1 Sorbent layers and sorbent layer systems for OPLC separations

Three geometric arrangements are used for chromatographic development in conventional TLC or HPTLC: linear, circular (radial), and triangular (anticircular). Depending on the application, all these developing modes can be performed in OPLC, and each has its own particular merits. In the linear developing mode, one-directional, and two-directional and two-dimensional developments are possible (68).
Contrary to circular OPLC technique, the linear development requires a special chromatoplate that is sealed at the edges. This prevents the eluent flowing off the chromatoplate in an unwanted direction. Linear migration of the eluent front in the OPLC chamber using the linear developing mode can be achieved by placing a narrow channel before the eluent inlet. The function of the eluent trough is to direct the eluent and to form a linear eluent front. In practice, a polyethylene or PTFE insert with an eluent trough is placed between the sorbent layer and the water cushion to protect the

![Diagram](image)

**Figure 13** Schematic drawing of chromatographic plates used in OPLC separations (36, 69).  
a, circular (transfusion); b, one-directional transfusion; c, two-directional transfusion; d, two-directional infusion; e, one-directional transfusion; f, two-dimensional infusion; g, parallel coupled multilayer; h, serial coupled multilayer; i, parallel/serial coupled multilayer.
cushion and to direct the eluent (68).

Figure 13 shows the modification possibilities of the chromatoplate (36, 69). It is obvious that for circular OPLC separation (a), it is not necessary to impregnate the edges of the chromatoplate and the eluent inlet is placed in the middle of sorbent layer. For one-directional development a plate sealed on three (b) or four (e) sides should be used. If two opposite sides (c) or all the four sides (d) of the chromatoplate are sealed and the eluent inlet is in the middle of the sorbent layer, the system is suitable for a two-directional separation with a large number of samples. For two-dimensional separation (f) in off-line systems, the four sides of the chromatoplate must be sealed beforehand and the seal opposite the actual inlet covered with a strip of filter paper, or an eluent outlet should be used in case of transfusion operation. For infusion separation (d, e, f) the layer is sealed on four sides and the outlet is absent or it is in close state (no overrun possibility).

For on-line OPLC separation two eluent directing troughs should be generated in the sorbent layer or in the PTFE insert cover plate. The eluent inlet trough directs the eluent along a linear front while eluent outlet trough collects the eluent at the end of chromatoplate connecting to detector system. The combination of several chromatoplates (g, h, i) during a single OPLC separation generates special advantages (e.g. a large number of samples). The introduction of the eluent to the multi-layer system is a critical matter and is performed by making a perforation in the chromatoplates, of a suitable size and shape, at the eluent inlet and/or outlet.
4.2 *Gas- and water-cushion experimental and commercial instruments*

The essential feature of the pressurized ultramicro chamber system is that the sorbent layer is completely covered with a flexible membrane under an external pressure so that the vapor phase above the layer is virtually eliminated (20-22). In this chamber system it is possible to optimize the flow velocity of the eluent by means of a pump. Gas was used for external pressure formation in the very early experimental chambers and because of high danger; it was changed to water cushion system. The principle of a pressurized ultramicro chamber made of polymethylmethacrylate and used mainly for circular separation is illustrated in Figure 14.

![Figure 14](image_url)

*Figure 14* Schematic drawing of the circular-type pressurized ultramicro chamber (33, 35).

1, water inlet; 2, developing solvent inlet; 3, pressure gauge; 4, screw fastener; 5, rubber O-ring; 6, sorbent layer; 7, support plate; 8, plastic foil cushion system; 9, polymethacrylate support block.

It follows from the principle of OPLC that low (2 to 5 bar), medium (10 to 30 bar), and high (50 to 100 bar or more) operating pressures can be used in this planar layer liquid chromatographic technique (47).

Based on experience gained with experimental pressurized chambers, the LABOR Instrument Works (Budapest, Hungary) developed CHROMPRES 10 and CHROMPRES 25, the first commercial pressurized ultramicro chambers.

In CHROMPRES 10 the cushion is pressurized up to a given external pressure with a liquid pump, while the eluent is fed by another pump, having a smaller performance (Figure 15a). In this chamber the maximum cushion pressure permitted is 1.0 MPa. It
can be used with plastic, aluminum, or glass chromatoplates up to 20 x 40 cm, coated with fine-particle (5-6 µm) or superfine-particle (2-3 µm) sorbent, although efficient separations can also be obtained on smaller chromatoplates (e.g., 10 x 10 cm and 10 x 20 cm). Special plastic frames permit the use of glass chromatoplates of different sizes.

**Figure 15** Chrompres 10 (a) and Chrompres 25 (b) OPLC systems (47, 68).

In CHROMPRES 25 chamber the maximum cushion pressure permitted is 2.5 MPa and the maximum size of layer is 20x20 cm (Figure 15 b). The optimum eluent front velocity is higher in a superfine-particle (2-3 µm) sorbent layer than in a fine-particle (5-6 µm) sorbent layer, and the increase of eluent front velocity means a higher solvent inlet pressure (47, 68). This chamber gives better conditions for such separations than the CHROMPRES 10.

Kaiser and Rieder (70, 71) established that high pressure in planar layer chromatography can be applied most easily in the circular and anticircular separation modes. They developed high-pressure planar liquid chromatography (HPPLC), which is theoretically and practically a circular version of OPLC at higher operating pressure.
(3.8-4.0 MPa) with special solutions. This technique exploits all the advantages of the circular technique and uses the experience gained in the field of circular HPTLC.

An instrument has been developed for OPLC separation by Witkiewicz et al. (72). In the instrument the eluent is fed to the chromatoplate, from below, by a syringe pump. Gas is used to apply external pressure to the chromatoplate. The instrument can be set up quickly and is easy to operate.

4.3 **OPLC instrument with cassette systems**

The last generation of OPLC instrument is an automated OPLC 50 system (developed by OPLC-NIT Ltd., Budapest, Hungary, distributes BIONISIS-OPLC SA, Le Plessis Robinson, France), includes the separation chamber and the liquid delivery system. The separation chamber has four main units as holding unit, hydraulic unit, tray like layer cassette and an attached drain valve (Figure 16). A microprocessor-controlled liquid delivery system is the heart of the liquid delivery. The pump heads work by means of a common drive, one for the eluent delivery, the other for the hydraulic liquid delivery. All parameters for single isocratic or stepwise gradient (max. 3 steps) development can be given and stored in the software of the delivery system. The automatic developments are absolutely repeatable and parameters can be stored during the working period after a simple fill-in the blanks. External pressure, eluent volume of rapid period and of development, eluent flow rate can be filled, while developing time

*Bold number refers to own result*
is automatically calculated. Automatic development can be managed under the PARAMETERS and DEVELOPMENT menu points. Every step can be monitored through the LCD display. External pressure can be selected between 0 and 5 MPa. The eluent is adjustable in the range of 10—10000 µl/min, and A, B, C solvent systems can be chosen for isocratic or step-wise gradient runs. The DEVELOPMENT menu automatically integrates separation steps of off-line or on-line developments. The process begins with external pressure formation. To ensure a straight front line, at the starting period of development (beginning the separation with dry sorbent layer) a rapid eluent flush is performed and, just before reaching the starting zone, the eluent devoted to separation starts traveling slower at constant (optimum) velocity. After delivering the full volume of eluent needed for the separation, an automatic termination is followed by the release of external pressure. An end-signal warns that the process is over, and the cassette can be pulled out.

Figure 17  Cassettes for transfusion and infusion OPLC separations (23, 29, 42, 69). a1, cassette for system rinsing; a2, cassette for two-directional run of 20x20 cm layers (only for infusion); b, cassette for one directional run of 20x20 cm layers; c, cassette for one directional run of 10x20 cm layers; d, cassette for one directional run of 5x20 cm layers; e, cassette for one directional run of 5x20 cm layers; 1, PTFE cover sheet; 2, eluent directing trough; 3, samples; 4, sorbent layer.
Linear, one- and two-directional, two-dimensional, circular cassettes can be used for infusion off-line and for transfusion off-line and on-line developments using analytical or preparative sorbent layer and the appropriate cassette (23, 29, 42, 69) (Figure 17).

The present state of OPLC includes the use of sample applicators of different types, scrapers for the removal of sorbent layers for conventional isolation of substances separated, eluent connections for the on-line method, staining systems for dramatization, densitometers for off-line quantitative evaluation, and detectors for on-line quantitative evaluation. In the case of isolation a connection of fraction collector is recommended.

### 4.4 One- and multi-channel experimental OPLC systems with flowing eluent wall (FEW) configuration

The new OPLC Purification Unit is a chamber having a built-in hydraulic pump to form 5 MPa of external pressure (Figure 18). For eluent delivery, an independent pump, e.g. an HPLC pump or the pump of an OPLC 50 instrument can be connected into the inlet tee of the chamber. The hydraulic unit of the separation chamber is equipped with two eluent inlet connections, one for sample injection and another one for the FEW.
formations. The outlet can be connected to a flow cell detector and/or a fraction collector. The cassette containing the sorbent layer can be inserted into the chamber.

The maximum migration distance between the inlet and outlet is 170 mm. This value corresponds to the distance between the eluent-directing troughs of the PTFE cover sheet of the cassette. The troughs for injection and collection are 178 mm and 184 mm long, respectively (27).

The four- and eight-channel FEW versions of the hydraulic unit were built into OPLC Purification Unit and the last one can be seen in Figure 19 (27, 28).

The last development is an integrated, computer controlled four-channel experimental system, called multiOPLC 4000 Work Station, includes a gradient pump, injector, a chamber with FEW configuration, UV-VIS detector and fraction collector working in parallel for all the four channels.

5 Operating steps and methods of OPLC

5.1 Transfusion, infusion and infusion-transfusion OPLC

Fully off-line OPLC has two operations the infusion and the transfusion one. In infusion mode the eluent is introduced into the totally closed layer (layer surface is

**Bold number refers to own result**
closed by external pressure, the layer edges are sealed on four sides and the chamber outlet is closed). The air originally contained the sorbent layer is continuously compressed during the process helping on the pore filling of particles. This yields a continuously reduced waviness of front of total wetness and an increased efficiency. The eluent introduction is finished when the inlet pressure reaches the pressure limit. The infusion process is suitable only for off-line developments (one- and two-directional, two-dimensional) without overrun and sorbent layer sealed on four sides should be used (23).

The transfusion operation corresponds to the classical (original) OPLC technique permitting the pass through for both the air and eluent (42). In this system both off-line and on-line operation as well as their combination is possible. In the case of on-line separation-detection the eluted air disturbs the first part of on-line detection. The infusion-transfusion operation is suitable for continuous development and serves better conditions for on-line detection flushing out the compressed air (23, 43).

5.2 Connection possibilities of operating steps of OPLC

OPLC is an instrumentalized version of planar layer liquid chromatography, and it is suitable for on-line as well as off-line sample application, separation, and detection, and their variations (partial off-line or partial on-line methods) (Figure 20).

In the fully off-line mode, all the principal steps of the chromatographic process are performed off-line (fully off-line OPLC).

In the on-line separation-detection mode with off-line sample application (a partial on-line process), the solutes are measured in the drained eluent by connecting a flowcell detector to the eluent outlet.

The process of on-line sample application-separation with off-line detection (a partial off-line process) in practice is less important.

The entire chromatographic process can be performed fully on-line (fully on-line OPLC) by connecting a loop injector to the eluent inlet and a flowcell (e.g. UV) detector to the eluent outlet, in much the same way as in HPLC (38, 41).
In analytical fully off-line OPLC, several samples can be processed in parallel. The technique offers further advantages, such as that only the spots or bands of analytical interest need to be assessed, quantitative evaluation can be repeated with various detection parameters including the application of specific reagent, and chromatogram spots or bands can be evaluated visually as well.

In OPLC systems the changes in the composition of eluent provide good possibilities for special separation modes, i.e., isocratic, gradient, and stepwise gradient. Automated OPLC 50 system generates a controlled separation process (29) and permits both analytical and preparative investigations.

In preparative fully off-line OPLC, after development the procedures of drying, scraping of the sorbent layer, elution, and crystallization are similar to conventional preparative TLC methods. However, in preparative off-line OPLC, the resolution is

**Figure 20** Schematic drawing of OPLC processes (23). (---) Off-line step; (——) on-line step. 1, chamber inlet; 2, chamber outlet; 3, outlet valve for the generation infusion and infusion-transfusion processes.
considerably increased and thick, fine-particle sorbent layers can also be used. It is possible to isolate only the components of interest from the sorbent layer.

When using a combined system, some sample components can be measured on-line (as in HPLC detection), although others that remain on the sorbent layer after the separation can be evaluated off-line by means of a densitometer. Figure 21 illustrates the basic elements of the combination of off-line and on-line OPLC (combined on-line/off-line OPLC).

Combining on- and off-line OPLC increases the efficiency of the OPLC system, providing approximately twice the spot capacity obtained by single systems because the spot and peak capacity are combined (24) (cf. Fig.11)

![Figure 21 Basic principles of the combination of off-line and on-line OPLC (69). A, schematic of off-line detection, indicates the effective range of the off-line separation; B, schematic of on-line detection, indicates the effective range of the on-line separation; C, combination of the effective ranges of off-line and on-line separations.](image)

Combination of a multi-layer system with a forced eluent flow complicates really to a certain extent the original simple and flexible TLC technique and also partly conventional OPLC. However, the result is an efficient and promising technique in the field of layer liquid chromatography which is applicable to analytical and preparative separations in various types of laboratories. **Parallel version of overpressured multilayer chromatography** (OPMLC) using two or more chromatoplates is very attractive because a large number of samples (50-100 or more) can be separated during one development (25) (see Fig. 13 g). The development of OPMLC exploits unique possibilities of the layer liquid system which are absent from column liquid systems.

*Bold number refers to own result*
Serial coupled OPMLC (called “long distance” OPLC) can be used for the elevation of the theoretical plate number and resolution alike as elaborated by Botz et al. (26). Long distance OPLC is a multi-layer development technique employing specially prepared chromatoplates. In a manner similar to the preparation of layers for linear OPLC development, all four edges of the chromatoplates must be sealed, and movement of the eluent with a linear solvent front can be ensured by placing a thin plastic sheet on the layer or by scraping a narrow channel in the sorbent for the solvent inlet. Several plates are placed on top of each other to extend the development distance (long distance OPLC). The end of the first (uppermost) chromatoplate has a slit-like perforation to enable the mobile phase to flow to a second layer where migration continues until the opposite end of the chromatoplate, here the chromatography can be continued on to a subjacent chromatoplate or the eluent be led away (see Fig. 13 h). Owing to the special arrangement of the prepared layers and the use of forced eluent flow, the mobile phase can travel through the stationary phases at optimum flow velocity. Of course, in this technical solution the development distance of chromatoplates can easily be increased to the extent desired. Another advantage of the method is the different sorbents can be used so that each part of a complex mixture can be separated on a suitable stationary phase.

It is obvious, that layer can couple in the parallel/serial mode as well (see Fig. 11i). This version is well-suited for efficient micro-preparative isolation.

5.3 Sample transfer by OPLC to HPLC separation

Analysis is rather difficult when the sample contains impurities in high concentration together with the components to be measured. This situation is typical in case of biological samples. The sample has to be purified in one or more steps before chromatographic analysis.

OPLC itself can also be used as a sample cleanup unit of multidimensional systems for separation and identification of components of complex mixtures. The efficiency of the HPLC separation can be improved by direct coupling it with OPLC transferring selected, preliminary separated components to the column (73). The schematic drawing of this OPLC-HPLC system can be seen in Figure 22. The system can be combined with
conventional TLC separation as well. The OPLC works as an interface unit transferring the preliminary separated components from the localized area of the sorbent layer. Figure 23 shows the variations.

4.4 One- and multi-channel OPLC separations in the sorbent layer segmented by flowing eluent wall

A new general concept has been developed for single-channel and multi-channel OPLC separations using a non-segmented sorbent bed and a flowing eluent wall (FEW) system for operational segmentation (27). According to this new concept, in the case of

![Figure 23](image)

**Figure 23** Scheme of sample cleanup procedure using a preseparation on the chromatoplate (73).
I, sample application; II, prewash of the impurities by conventional development; III, elution of the clean sample towards the column; 1, alpha front; 2, location of the isolated area of the layer;
A, easily removable fraction; B, fraction with Rf 0.2-0.8; C, trace enrichment.

![Figure 24](image)

**Figure 24** Scheme of one-channel OPLC separation using FEW formation only at inlet (a) and with FEW arrangement both the inlet and outlet sides(b) (27, 28).
single on-line sample application-separation and on-line and/or off-line detection, the sample as well as the eluent can be introduced into the same place of the sorbent layer (Figure 24). For mobile phase administration, a pump can be used. The mobile phase distributor (T) scatters the stream of eluent to the mobile phase line of the FEW and to the mobile phase line of sample injection, where sample is applied into the eluent stream by the injector. The distributor space of the eluent to form the FEW and the distributor space of sample application transfers and spreads separately the applied liquid connecting to the sorbent bed. These are close to each other and have small volume with a trace of flow resistance perpendicular to the development. The linear velocity of FEW part as well as the separation part is roughly the same. The velocity at the sides is distinct from the homogeneous velocity of the bulk. At the outlet side of a non-segmented sorbent bed, the sample and/or mobile phase collector space collects and transfers the effluent stream to the flow cell detector, which can be connected to a fraction collector for isolation purposes (Figure 24a). In order to eliminate the contaminants originated from the sealing material the separation line is collected separately from the FEW line (Figure 24b)(28). The collector space at the outlet has a small volume and low flow resistance, and it is perpendicular to the mobile phase movement.
In the case of parallel fully on-line separation the FEW can be used for the operating segmentation of non-segmented sorbent bed, detaching it into active and non-active parts regarding separation as can be seen in the Figure 25. Only mobile phase is introduced into the non-active part, while for the active part (separation) eluent and also the sample can be admitted; thus, the unsuitable part of the sorbent bed is excluded from the separation process. Accordingly, FEW can help in the elimination of edge effect of OPLC in case of single sample injection; moreover, it also can eliminate the sample mixing effect of neighboring lanes in the case of multi-channel separation process. If both the inlet and outlet connections have a trace of flow resistance, the flow in the sorbent bed is self-regulated.

**Figure 25** Scheme of four (a) and eight-channel (b) OPLC separation using multi-injection and FEW formation at inlet collection at outlet (28).
1, non-segmented sorbent bed; 2, sealed edges; 3 distributor space of mobile phase to form flowing eluent wall; 4, distributor space of sample application; 5, FEW part of sorbent bed; 6, separation part of sorbent bed (lane); 7, components separated; 8, sample collector space at outlet side; E, eluent; I, injectors; D, detectors; W, waste collected by FEW lines.

**Bold number refers to own result**
Figure 26 shows the difficulties of parallel sample application without the use of FEW in the case of the multi-channel version. The chromatogram of the parallel sample injection-separation clearly shows the side effect and the effect of sample mixing; moreover, the sample partly moves into the backside of the trough.

**Figure 26** Chromatogram of four-channel OPLC separation of dyes without FEW using 50 µL injection volume for four parallel channels (27). External pressure, 5 MPa; flow rate, 1 ml/min; eluent, toluene. 1, parallel injection troughs; 2, edge effect; 3, sample mixing effect of neighboring sample lanes; 4, back side effect.

Figure 27 shows, that all the effects are eliminated by means of FEW.

**Figure 27** OPLC separation of dye mixtures using single- and multi-channel injections with FEW (28). a, one-channel; b, four-channel; c, eight-channel
Figure 28 shows a fully on-line FEW-OPLC separation of commercial chamomile oil using the four-channel version. As can be seen, the components are eluted in parallel in spite of the different injection volumes.

The *multiOPLC 4000* Work Station was applied for parallel purification of complex synthetic samples (74).

**Figure 28** Fully on-line isocratic OPLC separation of chamomile oil using four-channel OPLC 50 system with FEW configurations (28). Eluent, hexane-ethyl acetate (90+10, v/v); flow rate, 1.5 ml/min; detection at 254 nm; chamomile oil, 20 mg/ml; a, 20 µl injection distributed to the 1st and 3rd channels, detection at the 1st lane, AUFS 0.5/0.5 V; b, 60 µl injection distributed to the 2nd and 4th channels, detection at the 4th lane, AUFS 0.5/1 V
THESES

1. Based on the ultramicro chamber a new forced flow planar layer liquid chromatographic technique, the overpressured layer chromatography (OPLC) has been developed integrating the advantages of TLC and the HPLC (e.g. optimal linear velocity generated by a pump, high number of parallel separations, applicability of specific reagents).

2. Basic conditions of the linear separations, such as the layer sealing, eluent introduction onto the layer close to the edge, eluent distribution for the linear front formation and a rapid eluent admission at the starting period of separation have been developed. The developed sealed layers and also the cover sheet having linear troughs ensure the easy use of the system. Based on the scientific knowledge’s cumulated different OPLC systems have been developed.

3. The relationships of the movement of the mobile phase have been revealed in the case of OPLC. In the linear transfusion OPLC (when the end of the layer is in open stage) the movement of alpha front, the front of total wetness generated by the pore filling of particles as well as the secondary fronts caused by eluent demixing produce straight relationship and their movements are proportional regarding the alpha front.

4. Based on the original off-line transfusion OPLC, a new version, the on-line OPLC has been developed by building into the chamber an outlet connection. If the system is equipped with injector at the inlet and a flow cell detector at the outlet it is well suited for on-line and/or off-line combinations of chromatographic operating steps, such as sample application, separation, detection and isolation. The fully on-line OPLC operation corresponds to the HPLC configuration. A special combination of on-line and off-line detection in OPLC (combined on-line/off-line OPLC) yields two times better spot/peak capacity comparing the values with off-line or on-line separation-detection.

5. In order to reduce the efficiency loss of the conventional transfusion OPLC caused by the front of total wetness, infusion as well as infusion-transfusion operations have been developed. In infusion OPLC the outlet of the chamber is closed and the eluent is introduced into the totally closed sorbent layer. It is suitable only for off-line separation without overrun. The infusion-transfusion operation allows continuous and on-line developments, and serves better conditions for on-line detection than transfusion OPLC, flushing out the compressed air (no bubbles).

6. The efficiency parameters of OPLC—theoretical plate height (H), theoretical plate number (N), spot and/or peak capacity (n), resolution (R_s)—have been studied using different separation conditions and different versions of OPLC. The effect of development distance on H shows the principle of OPLC, because of its value is roughly constant along the layer contrary to the TLC, where an efficiency loss exists over a distance. The differences between TLC and OPLC are higher in the case of use of fine particle sorbent layer. The elevation of external pressure on the surface of sorbent layer results in more efficient separation in off-line OPLC and also broader optimum range of linear velocity.
Such effect was not found in the case of on-line OPLC. Comparing the effect of different operating modes on efficiency it was found that fully off-line OPLC yields better efficiency, than fully on-line process.

7. The combination of OPLC with HPLC is especially effective for cleanup and separation. The cleanup step can be carried out in OPLC prior to the HPLC separation. Another solution of combination is, when the pre-separation is fulfilled in a normal tank and OPLC works as an interface transferring separated components from the localized area of the layer to the HPLC system.

8. A new OPLC separation procedure has been developed for single- and multi-channel separation using a non-segmented sorbent bed and flowing eluent wall (FEW) for operating segmentation. The FEW detaches the sorbent bed into active and non-active parts regarding separation during the process. Only mobile phase is introduced into the non-active part, while, for the active part, eluent and also the sample can be admitted, thus the non-homogeneous part of the sorbent bed is excluded from the separation process. The FEW helps the elimination of the edge effect of overpressured layer chromatography (OPLC) in case of single sample injection and abolition of the sample mixing effect of neighboring lanes in the case of a multi-channel separation process. In the case of dirty samples, the one-channel FEW-OPLC system is well suited for quick isolation in different preparative ranges using preparative chromatoplates. The multi-channel solution will be a tool for high throughput analysis using efficient fine, superfine, or monolithic layers. The four-channel version can be applied for high throughput multi (parallel) analysis as well as micro- and semi-preparative parallel isolation using efficient analytical or preparative layers. The FEW provides the possibility for real multi-channel liquid chromatographic separation on a non-segmented sorbent bed.

**PRACTICAL MOMENTS OF THE RESULTS**

Based on the patent system and know how’s led to the fundamental renewal of the planar layer liquid chromatography, TLC. The technical solutions of analytical and preparative off-line and on-line OPLC (developed simple and automated systems, sealed layers and layer systems) demonstrate the significant improvement of the technology. The present instrument versions of OPLC (especially the automated OPLC 50) and the ready to use sealed layers having different phases offer the possibilities of the efficient best ever analysis and isolation of synthetic and natural compounds in the field of planar layer liquid chromatography. Owing to the high flexibility of OPLC, contrary to other layer liquid chromatographic techniques, it gives a special exploitation in the field of chemical-biochemical analysis and isolation.

The practical exploitation of the results means also the fabricated and the sold instruments in Hungary as well as abroad.

The validated methods help on the spreading of the OPLC technology. Over ten validated methods are only in Richter Gideon Ltd (Budapest, Hungary) in the daily routine work.
The newly developed one-, four- and eight-channel flowing eluent wall systems (FEW) give further possibilities for the parallel on-line analysis and micro- and semi-preparative works, which will be especially attractive in the field of combinatorial chemistry and also in the field of the natural compound research.

The theory and practice of OPLC technique is already an educational material of universities.

REFERENCES


**Bold number refers to own result**


*Bold number refers to own result*