significantly by employing the sequential technique.
9. On line preparative separation of 50–500 mg samples can generally be applied in a single chromatographic run.

**Comparison and Outlook of FFPC Methods**

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

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

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


**Further Reading**


used, migration of the mobile phase is also promoted either by application of external pressure (overpres-
sured layer chromatography (OPLC)) or by use of centri
fugal force (rotation planar chromatography (RPC)). (For more information see the previous entry Modes of development: Forced flow, OPLC and cen-
trifugal.) The enhanced efficiency obtained by use of
the optimum mobile phase velocity is independent of
layer thickness and of the type of forced flow applied.

**Parameters of PPC Separation**

One of the most important experimental variables in
PPC is the vapour space, because the separation pro-
cess occurs in a three-phase system of stationary,
mobile, and vapour phases, all of which interact until
equilibrium is reached. The most important factors
which might influence a PPC separation are shown in
Figure 1.

**Stationary Phase**

Although alumina, cellulose, and C₂ and C₁₈ reversed-
phase pre-coated preparative plates are available,
silica has been most widely used by far. The silica
materials commonly used for PPC have coarse par-
ticle sizes (average ~ 25 μm) and their distribution
range (between 5 and 40 μm) is also wide; Figure 2

**Figure 1** The principal factors affecting preparative layer chromatography.

**Figure 2** Particle sizes and particle-size distributions of silica stationary phases.
compares the quality of pre-coated analytical (TLC and HPTLC) and preparative plates, and that of silica for TLC. The advantage of making one’s own preparative plates is that any desired thickness (< 10 mm) or layer composition (incorporation of salts, buffers, etc.) becomes feasible. Pre-coated 20 cm × 20 cm or 20 cm × 40 cm preparative plates with layer thicknesses 0.5, 1.0, 1.5, and 2 mm have the advantage of much higher reproducibility. It is generally accepted that higher resolution can be achieved on a thinner preparative layer (0.5–1.0 mm) and the resolution is much more limited on a higher capacity (1.5–2 mm) layer. The loading capacity of a preparative layer increases with the square root of the thickness, practically without loss of separating power so that as a rule of thumb, the loading capacity of a 0.5 mm layer is approximately half that of a 2 mm layer.

Preparative plates are commercially available with or without preadsorbent zones. The preadsorbent zone (generally 4 cm width) serves as a holding zone for the sample until development begins. Soluble compounds migrate with the mobile phase front through the preadsorbent zone and are concentrated in a narrow band as they enter the chromatographic layer, thus improving the resolution. The materials used to manufacture these concentrating zones are kieselguhr or inert silica. Resolution can also be significantly increased by using a layer-thickness gradient that contains a wedge-shaped silica layer ranging in thickness from 0.3 mm at the bottom to 1.7 mm at the top, with an adjacent 700 μm preadsorbent layer for sample application. The cross-sectional area traversed by the mobile phase front increases during migration through the tapered layer, so the cross-sectional flow per unit area is highest at the bottom of the layer and decreases towards the solvent front. As a result the lower portion of a zone moves faster than the upper portion, keeping each component focused in a narrow band. Theoretical separations on a preparative plate, without and with preadsorbent, and compared with a tapered plate, are depicted in Figure 3A–C. The improved resolution as a result of the greater local mobile phase velocity clearly suggests the wider use of a preadsorbent layer or a layer-thickness gradient if at all possible.

**Mobile Phase**

The separations can be started in saturated or unsaturated chromatographic tanks. However, on starting the separation in an unsaturated chamber the chromatographic tank becomes saturated during the development because of the long separation time (1–2 h). If saturated chromatographic chambers are used, the optimized analytical mobile phase may be transferred unchanged to PPC. Because the particle sizes and size distribution of sorbents for preparative purposes are larger, and the plates are overloaded with the compounds to be separated, inferior separation is invariably achieved on preparative plates. This means that a successful preparative separation will need an optimized mobile phase.

The ‘PRISMA’ mobile phase optimization system enables not only optimization of solvent strength and mobile phase selectivity, but also transfer of the optimized mobile phase between the different planar chromatographic techniques. The system is based on the solvent classification by Snyder, who classified more than 80 solvents into eight groups for normal phase (NP) chromatography according to their properties as proton acceptors ($X_a$), proton donors ($X_d$), and their dipole interactions ($X_n$). Because

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**Figure 3** Comparison of separations on different preparative plates. (A) Pre-coated chromatographic plate without concentrating zone, (B) pre-coated chromatographic plate with concentrating zone, (C) pre-coated chromatographic plate with layer-thickness gradient.
$X_a + X_d + X_n = 1$, the solvents can be characterized by two of them (e.g. $X_a$ and $X_d$), and so a new definition was introduced – the individual selectivity value ($S_i$), which is the ratio of $X_a$ to $X_d$. The individual solvent strengths ($S_i$), selectivity values, and viscosities of the solvents most often used for normal phase PPC, are listed in Table 1.

For characterization of multi-component mobile phases the total solvent strength ($S_T$) can be defined as the sum of the $S_i$ of the components, weighted by multiplication by their volume fraction. The total selectivity factor ($S_V$) can be also calculated similarly to the $S_T$ value.

The volatility of the individual solvents must be also considered during the optimization process, otherwise several problems can arise in subsequent steps (elution of the compound from the stationary phase, evaporation of the solvent). It also precludes the use of, e.g. acetic acid as a component of the preparative mobile phase, because of the possibility of chemical degradation during concentration of the isolated compounds. Multicomponent mobile phases should not be used repeatedly, whereas single-solvent mobile phases can be used repeatedly until they become contaminated.

Figure 4 shows the transfer possibilities of the mobile phase, where thick lines indicate direct transfers. The thin lines indicate transfers which are also possible, but the solvent strength and selectivity must generally be changed. The dashed lines indicate direct transfer possibilities for fully online separation processes.

### Table 1 Selected solvents for normal phase PPC separation

<table>
<thead>
<tr>
<th>Selectivity group</th>
<th>Solvents</th>
<th>$S_i$</th>
<th>$S_V = \frac{X_a}{X_d}$</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>n-Hexane</td>
<td>0</td>
<td>0.01</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Methyl-t-butyl ether</td>
<td>2.7</td>
<td>3.50</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Diethyl ether</td>
<td>2.8</td>
<td>4.08</td>
<td>0.23</td>
</tr>
<tr>
<td>II</td>
<td>n-Butanol</td>
<td>3.9</td>
<td>3.11</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>4.3</td>
<td>2.74</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>5.1</td>
<td>2.18</td>
<td>0.52</td>
</tr>
<tr>
<td>III</td>
<td>Tetrahydrofuran</td>
<td>4.0</td>
<td>1.90</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Methoxyethanol</td>
<td>5.5</td>
<td>1.59</td>
<td>0.95</td>
</tr>
<tr>
<td>V</td>
<td>Dichloromethane</td>
<td>3.1</td>
<td>1.61</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>1,1-Dichloromethane</td>
<td>3.5</td>
<td>1.43</td>
<td>0.79</td>
</tr>
<tr>
<td>VI</td>
<td>Ethyl acetate</td>
<td>4.4</td>
<td>1.48</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Methyl ethyl ketone</td>
<td>4.7</td>
<td>1.59</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Dioxane</td>
<td>4.8</td>
<td>1.50</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>5.1</td>
<td>1.52</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Acetonitrile</td>
<td>5.8</td>
<td>1.15</td>
<td>0.39</td>
</tr>
<tr>
<td>VII</td>
<td>Toluene</td>
<td>2.4</td>
<td>0.89</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>2.7</td>
<td>0.72</td>
<td>0.69</td>
</tr>
<tr>
<td>VIII</td>
<td>Chloroform</td>
<td>4.1</td>
<td>0.61</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>10.2</td>
<td>1.00</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* Preferred solvent.

The optimized TLC mobile phase can be transferred from unsaturated chromatographic tanks to analytical OPLC and U-RPC without modification of the selectivity. If the tank is saturated, the optimized mobile phase can be transferred to M-RPC. Transfer of the corresponding preparative methods (OPLC, U-RPC, M-RPC) from analytical normal-phase FFPC can be performed directly with the same mobile phase. Modification of the mobile phase used in the unsaturated TLC tank is necessary for transfer of preparative N-RPC or S-RPC separations. Direct mobile-phase transfer is also valid from OPLC and U-RPC to C-RPC. With the characterization of the different saturation grade of chromatographic chambers, excellent mobile phase transfer between analytical and preparative planar chromatographic methods and analytical HPLC can be achieved.

### Vapour Phase

The selection of the vapour space is a variable offered only by planar chromatography. Three important factors have the greatest influence on this parameter – the chamber type, the ratio of the surface of chromatographic plate to the chamber volume, and the temperature.

Basically, one can distinguish between normal (N) and sandwich (S) chambers. In the conventional N-chamber there is a distance of more than 3 mm between the layer and the wall/lid of the chromatographic chamber. If this distance is smaller, the cham-
ber is said to have the S-configuration. Both types of chromatographic chamber can be used for unsaturated or saturated systems, and the chambers used for FFPPC separation can be also assigned to the earlier mentioned two categories.

Rectangular glass N-chambers are most frequently used for classical PPC (CPPC). Starting the separation with unsaturated chromatographic tanks generally gives higher \( R_F \) values for NP systems because of the evaporation of the solvents from the surface of the layer. Disadvantages of using unsaturated tanks are that it can result in a concave solvent front, leading to higher \( R_F \) values for solutes near the edges; the reproducibility of this effect and of the \( R_F \) values can be poor. If the layer is placed in the chamber immediately after introduction of the mobile phase, separation starts in an unsaturated system which will become progressively more saturated during the course of the separation (Figure 5A). A chamber is saturated when all components of the solvent are in equilibrium with the entire vapour space before and during the separation. A rectangular glass tank (N-chamber) with inner dimensions of 21 cm \( \times \) 21 cm \( \times \) 9 cm is most frequently used. These tanks can be used for development of two 20 cm \( \times \) 20 cm preparative plates with 50–100 mL mobile phase. The chamber must be lined on all four sides with thick filter paper (Figure 5B) thoroughly soaked with the mobile phase (by shaking) and must stand for 60–120 minutes to become saturated with the vapour phase. Each plate must lean against a side wall, so the plates do not touch each other. The advantages of saturated tanks are that the ‘\( x \)’ front is much more regular and that the separation efficiency is higher for a development distance of 18 cm.

Of the different FFPPC techniques, normal chamber RPC (N-RPC), in which the layer rotates in a stationary N-chamber, belongs to this category. Because of extensive evaporation in the extremely large vapour space, this chamber is practically unsaturated.

S-chambers are very narrow unsaturated tanks, the plate with the layer is usually sandwiched with a glass cover plate (Figure 5C). Saturation can be established with a facing chromatographic plate that has been soaked with the mobile phase (Figure 5D). Part of the stationary phase of the plate to be developed is removed by scraping, so that initially the mobile phase can only reach the level of the facing plate. After sorptive and capillary saturation of this plate, the depth of the mobile phase is increased to start the separation. The U-chamber is a special variety of S-chamber in which the vapour space is reduced (Figure 5E). All basic development modes enable both equilibration before development and a choice of flow rates for separation.

The chambers used for preparative 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. M- and U-chambers in RPC also belong to the S-chamber type. The difference between these two chambers is that the former is rapidly saturated, whereas in U-RPC the lid of the rotating chamber is placed directly on the chromatographic plate so that there is practically no vapour space and the chamber must be regarded as unsaturated.

The ratio of the surface area of the chromatographic plate to the chamber volume plays a role only if the separation is started in unsaturated tanks. The higher this ratio, the more unsaturated is the chromatographic chamber.

In saturated chromatography chambers the temperature does not exert a great influence on preparative separations. With unsaturated tanks the composition of the mobile phase plays a more pronounced role and it is important to note that temperature control is now important if separations are to be reproducible.
Figure 6  Calculation of the effect of chamber saturation on the separation.

For characterization of chamber saturation, a test-dye mixture can be developed with dichloromethane. If the $hR_F$ values of the dye mixture are the same in different chambers on a given stationary phase, the extent of chamber saturation is identical. For comparison, the $hR_F$ values obtained from different chambers can be depicted in a coordinate system. The $hR_F$ values of any given system are plotted along the $y$ axis and those from the system being compared along the $x$ axis. If the chamber saturation is identical, a linear relationship is obtained, and $\tan \alpha$ for the line is 1. If the $hR_F$ values obtained in the second system were smaller, the vapour phase was more saturated and $\tan \alpha > 1$, or $\alpha > 45^\circ$. Conversely if $\tan \alpha < 1$, or $\alpha < 45^\circ$, the vapour phase in the second system was less saturated. These possibilities are illustrated by results A and B in Figure 6. With this approach the chamber type can be characterized for a certain separation without specification of the vapour phase. The technique also provides guidelines for the transfer of mobile phases between the different planar chromatographic methods.

Development Mode

The ascending mode, in which the mobile phase moves up the plate, is most frequently used with a maximum separation distance of 18 cm. The angle at which the plate is supported during development affects the rate of development and the shape of the bands. As the angle of the plate decreases towards the horizontal development mode, the flow of the mobile phase increases, but so also does spot distortion. An angle of 75° is recommended as optimum for development. Because descending development has no significant advantages in terms of resolution, it is rarely used.

The advantages of circular development of compounds in the lower $R_F$ range are well known, but it has not been accepted for preparative separations, because the mobile phase velocity would be too slow. However, it is possible to start development not directly from the centre, but from a circle of 2 cm radius, i.e. the mobile phase inlet is not a point, but a circle. Because the size of the mobile phase inlet and the velocity of the mobile phase are related linearly, a relatively high mobile phase velocity can be achieved over a separation distance of 8 cm. Recently a new device has been described which enables a suitable mobile phase velocity to be used in the circular development mode (Figure 7). A solvent reservoir

Figure 7  Schematic diagram of a circular preparative chromatography chamber. 1, glass plate; 2, chromatoplate; 3, support block; 4, magnet; 5, Teflon ring; 6, solvent reservoir; 7, quartz glass cover plate; 8, Teflon ring; 9, solvent.
made of steel and a silicone sealing ring are placed on the layer and fixed by a magnet located below the chromatographic plate. To start the separation, adsorbent is scratched from the centre of the plate and the recess produced filled with mobile phase. The device can be used for different types of chamber (N, UM). The entry of sample and mobile phase is regular over the whole cross-section of the preparative layer, irrespective of whether the sample is applied as a liquid or a solid. The device ensures rapid, efficient separation with all the advantages of circular development. The resolution is significantly higher than that obtained from linear development. With the UM chamber the glass cover plate is placed directly on the surface of the chromatographic plate. In the N chamber, the cover plate is placed on a 19 cm diameter metal ring, the height of which can be varied between 0.5 and 2 cm, depending on the type of chamber applied. To start development the solvent reservoir is filled with the appropriate mobile phase and the level of this is kept constant by applying a constant hydrostatic pressure by means of a second reservoir. To stop development the inlet from the second reservoir is turned off.

Anticircular development is accepted in analytical TLC for increasing resolution in the higher $R_f$ range. Because a special device is necessary for such separations, this development mode is rarely used.

Although the different types of multiple development (MD) are rarely used for preparative purposes, the advantage of the method should be understood. In MD the first development length is the shortest and subsequent developments are performed over longer development distances. The last migration distance is the longest and corresponds to the useful development length of the chromatographic plate; it also depends on the nature of the mobile phase. The removal of the mobile phase between development steps is performed by careful drying of the plate. The dried layer is returned to the development chamber for repeated development under the same chromatographic conditions as for earlier development steps. The most important aspect of MD techniques is the spot-reconcentration mechanism. In each development step the solvent front first contacts the lower part of the chromatographic zone formed in the previous chromatographic step. The molecules at this part of the zone start moving with the mobile phase toward the molecules in the upper part of the chromatographic zone – those still ahead of the solvent front. As the mobile phase front reaches the upper part of the zone, the narrow band developed as a result of the zone reconcentration mechanism migrates and broadens by diffusion in the mobile phase, as in conventional planar chromatography.

In terms of development distance and mobile phase composition, MD techniques can be classified into four basic categories – UMD, IMD, GMD, and BMD (Figure 8). Unidimensional multiple development (UMD) is the repeated development of the chromatographic layer over the same development distance ($D$) with the same mobile phase (same values of $S_{T1}$ and $S_{V1}$). In the modification of UMD known as incremental multiple development (IMD) re-chromatography is performed over increasing development distances ($D_1 \rightarrow D_3$) with the same mobile phase (same values of $S_{T1}$ and $S_{V1}$). In gradient multiple development (GMD), successive chromatographic development steps are performed with a change in solvent strength and selectivity ($S_{T1}, S_{V1}; S_{T4}, S_{V4}$) over the same development distance ($D$ is constant). GMD is required for analysis of multicomponent mixtures spanning a wide polarity range. The most complex multiple development technique is bivariate multiple development (BMD), in which development distance and mobile phase composition are varying simultaneously ($D_1, S_{T1}, S_{V1}; D_4, S_{T4}, S_{V4}$) during successive chromatographic developments. Needless to say, the solvent strength and selectivity of the mobile phase can be changed independently of each other. For the analysis of less complex mixtures of wide polarity range, the preferred technique is BMD with a mobile phase gradient of decreasing solvent strength, when the final chromatographic separation can be detected as a single chromatogram. In BMD the shortest development is performed first with the mobile phase of strongest solvent strength; the chromatographic distance is increased and the solvent strength reduced during successive steps of the chromatography, until finally the last development step is performed over the longest development distance with the weakest mobile phase.

**Separation Distance**

The separation distance depends on the dimensions of the plate, the mode of development, the particle size, and the size distribution. The last property cannot be influenced by the user of pre-coated chromatoplates. Because in classical PPC capillary action is effective only for plates up to 20 cm in length, the maximum separation distance is 18 cm. For circular development the separation distance is 8–9 cm; in anticircular mode this distance is 8 cm maximum. Despite the short separation distance, correct selection of mobile phase and development mode can give high resolution.

The separation pathway in CPPC can be increased by use of a sequential technique in which the mobile phase supply to the plate is fully variable in time and
The principle of this technique is that the mobile phase velocity is much higher at the beginning of separation than later. After an initial separation, the layer is carefully dried, and the mobile phase applicator is placed between two separated zones, irrespective of whether the same or a different mobile phase is used. The supply of mobile phase can be stopped at any time to transfer it directly to the area of the compound zones to be separated. This always gives the highest initial velocity of the mobile phase, which substantially shortens the analysis time. The sequential technique for preparative separations can be performed with a special device—the Mobil-$R_F$ chamber.

The separation pathway in S-RPC becomes theoretically unlimited as a result of a special combination of the circular and anticircular development modes. With this technique the mobile phase can be introduced onto the plate at any desired place and time. The solvent application system works in circular mode and with the aid of capillary action against the reduced centrifugal force (anticircular mode). Generally the circular mode is used for the separation and the anticircular mode for pushing the substance zones back to centre with a strong solvent. After drying the plate at a high rotation speed, the next development with another suitable mobile phase can be started. S-RPC is selected when the separation problem...
cannot be solved with a single mobile phase, but optimized mobile phases are available for the separation of the individual compounds. Also the sequence technique can be employed for fast elution of previously separated compounds with a solvent of high strength.

**Sample Application**

Sample application is one of the most critical steps in PPC. The sample can be applied either offline or online. In offline mode the mobile phase comes into contact with the stationary phase only when sample application is complete; the separation is always started with a dry layer. This mode of application can be used for all PPC methods, irrespective of the driving force (capillary action or forced flow). In online application the sample is dissolved and the stationary phase is always wetted with a solvent – generally it is equilibrated with the starting composition of the mobile phase. This application mode is only possible when forced-flow techniques are used.

**Offline Liquid-Phase Sample Application**

The preferred method of placing a sample on a preparative layer is to apply it as a narrow streak across the plate. It is highly desirable to have the streak as straight and narrow as possible. With practice it is possible to streak a plate correctly by hand, using a syringe; the use of a Teflon tip on the end of the syringe has the advantage that no mechanical disturbance of the layer occurs. On applying a large amount of sample, the streak can be focused simultaneously and concentrated to a relatively thin line by the sequential technique, especially with nonpolar samples.

Application of a continuous streak can be automated. Most available applications can give a sample zone < 3–4 mm wide and up to 200 mm long for preparative separations. When the layer is not overloaded with sample it is generally accepted that the streak should be applied across the plate 2 cm from both edges. These areas are left free, partly because of the edge effect, which can cause migration of the mobile phase to be faster or slower at the edge than on the centre of the plate. For separations of extremely large amounts, the sample can be applied over the whole width of the plate. Otherwise migration of the mobile phase would be faster in the sample-free areas, resulting in poorer resolution.

When using pre-coated preparative plates with concentrating zones, the quality of streaking is not very important because the sample is applied to a practically inert zone. Pre-coated preparative layers with concentrating zone can be successfully used for PPC and for linear OPLC.

For preparative rotational planar chromatography (RPC), a concentrating zone can be self-prepared. The layer is removed by scraping to furnish a larger inner circle; a slurry with inert material is then cast in this circle. After final drying and scraping, the layer has a preadsorbent zone of approximately 2 cm. For C-RPC separations, after filling the planar column with the selected stationary phase, the last 1 cm can be filled with a deactivated sorbent.

Offline liquid-phase sample application can be used for practically all micropreparative and preparative PPC methods. The only exception is C-RPC, where the solvent for dissolution of the sample is not identical with the mobile phase. The planar column prevents elimination of the sample solvent. The separation may consequently be distorted, especially if the solvent has a higher solvent strength than the mobile phase.

**Offline Solid-Phase Sample Application**

Preparative solid-phase sample application is especially useful whenever a large amount of sample must be applied and/or the sample is soluble in nonvolatile solvent only. The sample must be dissolved in a suitable solvent and mixed with approximately 5–10 times its weight of deactivated sorbent. The sorbent with the adsorbed sample is dried and then introduced into a layer which has to be prepared to accept it. A 180 mm × 5 mm channel with a U-shaped profile can be scratched from the stationary phase. After removal of the sorbent from the channel, the prepared sorbent with the adsorbed sample is placed in the channel and pressure is applied to ensure optimum contact between the stationary phase of the chromatographic plate and the applied sample. Offline solid phase sample application is useful not only for conventional PPC but also for linear OPLC and C-RPC separations. In OPLC the prepared plate is placed horizontally in the OPLC chamber and the separation can be started with a relatively high inlet pressure. Because of the forced flow, any possible lack of suitable contact has no effect on the efficiency of the separation. With C-RPC the column must first be filled with stationary phase, then with the solid sample. Because of the centrifugal force no lack of suitable contact can occur.

**Online Sample Application**

In FFPPC the sample can also be applied to a wetted stationary phase, preferably to a plate equilibrated with mobile phase. This mode of application can be used not only for the various methods of preparative OPLC and RPC but also for semipreparative purposes. For plates without a concentrating zone and
online sample application, the separation time is increased because of the longer separation distance. When using the plates with concentrating zone, the effect of the reduced separation distance (2.5–4 cm) is compensated for by the efficiency of the concentrating zone. Because of these results, preparative separations with online sample application and plates with concentrating zones gave practically the same resolution in a shorter time than with the use of offline sample application on plates without concentrating zones. The mode of sample application has no significant influence on the resolution and is independent of the type of plate (analytical, preparative, with or without concentrating zone).

**Location and Removal of Separated Compounds**

After the preparative chromatographic plate has been developed and the mobile phase evaporated, the separated bands must be located and the desired compounds removed from the plate. If the compounds of interest are coloured, their position on the layer can be located under white light. If they are fluorescent, or become so after post-chromatographic derivatization, their position on the layer can be determined under UV light. Conversely, a PPC plate containing a fluorescent material will indicate the separated compounds as dark zones on a bright background when examined under UV light. Pre-coated plates containing 254 nm or 365 nm fluorescent indicators should be used if possible because they provide a mode of detection which is generally nondestructive.

If the compounds themselves are not visible or fluorescent, detection can be performed by use of iodine vapour or by use of destructive reagents (e.g. vanillin–sulfuric acid). If such a reagent is used for detection of the separated compounds, a vertical channel must be scraped in the layer about half a centimetre from the edge of the streak. After covering the major portion of the layer with a suitable glass plate, the part of the layer which is not covered is sprayed, and thus serves as a guide area. If heating is necessary for detection, the sprayed portion of the plate must be detached from the rest, by use of a glass cutter, because heating the developed preparative plates may lead to decomposition of the compounds of interest.

After location of the desired compound, the subsequent steps are: (1) mechanical removal of the adsorbent zone, (2) extraction of the compound from the stationary phase with a suitable solvent, (3) separation from the residual adsorbent, and (4) concentration of the solvent. The areas of the layer containing the compounds of interest are then scraped off cleanly down to the glass with a suitable scraper. One of the best methods is to put the adsorbent with the compound to be extracted in an empty receptacle containing a sintered glass filter to retain the adsorbent and to extract the compound with a solvent and the aid of vacuum.

The substance should be highly soluble in the solvent or solvent mixtures used for extraction; the solvent should also be as polar as possible. Chloroform is widely used for nonpolar substances, and ethanol or acetone for polar compounds. If water is the chosen solvent, it should be removed by lyophilization. Because silica is significantly soluble in methanol, this solvent should be avoided. The mobile phase used for the separation is highly recommended also for extraction. As a rule of thumb the volume of solvent (\( V_{\text{solvent}} \)) required when the chromatographic mobile phase is chosen for extraction is as in equation [1]:

\[
V_{\text{solvent}} = 10 \times (1.0 - R_F) \times V_{\text{scraped}} \quad [1]
\]

It should be noted that the longer the substance is in contact with the adsorbent, the more likely decomposition is to occur. Once the solution of the compound to be isolated is obtained (free from adsorbent) the extract must be evaporated to dryness. The evaporation temperature should be as low as possible, to avoid decomposition.

**Selection of Appropriate PPC Method**

All types of PPC can be used for purification and isolation in the micropreparative and preparative range. As a rule of thumb, if the sample contains more than five substances, up to 10 mg of sample can be separated by a micropreparative method and up to 500 mg by a preparative method. If the sample contains fewer than five substances, these values can be increased to 50 and 1000 mg.

Conventional PPC can be used successfully, if: (1) no more than five compounds must be separated, (2) the compounds to be isolated are distributed over the whole \( R_F \) range and are present in more or less the same amounts, and (3) the total amount of sample does exceed 150 mg. This is the simplest and therefore the most widely used method.

Online OPLC can be used for the separation of five to seven compounds in amounts up to 300 mg. The use of centrifugal force for online purification and isolation is the oldest forced flow method. Generally, a 15 µm particle size stationary phase is used with all the advantages of the free selection of the size of the vapour space and the development mode. Up to ten compounds in amounts up to 500 mg can be isolated using the appropriate RPC method.
Comparison and Outlook of PPC Methods

The principal differences between classical PPC, OPLC, and RPC are summarized in Table 2 which lists the generally accepted characteristics of the methods. As is apparent, the major difference between the methods is the nature of mobile phase migration. Better resolution can always be achieved by use of forced-flow techniques (OPLC, RPC) because the mobile phase velocity is nearer to the optimum; the use of online separation eliminates the need to scrape the separated compounds from the plate and means that all the compounds migrate over the whole separation distance. This enables connection of a flow detector, recorder, and fraction collector. These techniques require more sophisticated instrumentation. Unfortunately, the particle size and size distribution of pre-coated plates for OPLC are at present inadequate for this preparative technique.

Modern online forced flow methods enable not only micropreparative (OPLC) and preparative (RPC) separations, but – using appropriate split systems – also the hyphenation of these methods with different spectroscopic techniques like diode-array detection (DAD), FTIR, MS, and NMR, as is apparent from Figure 9. In this way not only isolation but also structure elucidation can be carried out in a single operation process.

Figure 9 Combination of preparative FFPC and modern spectroscopic methods.

<table>
<thead>
<tr>
<th>Viewpoint</th>
<th>CPPC</th>
<th>OPLC</th>
<th>RPC (N-, M-, U-, S-, C-RPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration of mobile phase</td>
<td>Capillary action</td>
<td>Pressure</td>
<td>Centrifugal force/capillary action</td>
</tr>
<tr>
<td>Layer/column</td>
<td>Pre-coated</td>
<td>Pre-coated</td>
<td>Self-prepared/filled</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>Silica, RP-2, RP-18</td>
<td>Silica</td>
<td>All available</td>
</tr>
<tr>
<td>Particle size of stationary phase</td>
<td>$5 \mu m &lt; x &lt; 40 \mu m$</td>
<td>$5 \mu m &lt; x &lt; 40 \mu m$</td>
<td>$5 \mu m &lt; x \leq 15 \mu m$</td>
</tr>
<tr>
<td>Layer thickness</td>
<td>0.5, 1, 2 mm</td>
<td>0.5, 1, 2 mm</td>
<td>1–4 mm</td>
</tr>
<tr>
<td>Volume of stationary phase</td>
<td>Constant</td>
<td>Constant</td>
<td>Increasing (constant)</td>
</tr>
<tr>
<td>Vapour space</td>
<td>Normal tank</td>
<td>None</td>
<td>Variable (N, M, UM, none)</td>
</tr>
<tr>
<td>Separation distance</td>
<td>18 cm</td>
<td>18 cm</td>
<td>12 cm (unlimited)</td>
</tr>
<tr>
<td>Separation mode</td>
<td>Linear (circular)</td>
<td>Linear (circular)</td>
<td>Circular (circular–anticircular)</td>
</tr>
<tr>
<td>Isolation</td>
<td>Offline</td>
<td>Online (offline)</td>
<td>Online</td>
</tr>
<tr>
<td>Typical amount of sample</td>
<td>50–150 mg</td>
<td>50–300 mg</td>
<td>50–500 mg</td>
</tr>
<tr>
<td>Number of compounds</td>
<td>2–5</td>
<td>2–7</td>
<td>2–12</td>
</tr>
</tbody>
</table>
The greatest flexibility with regard to choice of stationary phase, particle size, layer thickness, and chamber type is provided by RPC. Because of the availability of suitable vapour phases and combination of development modes, RPC offers the greatest separating power both in terms of the amount of sample and number of compounds to be separated.

It can be stated that PPC covers a special range of preparative separations. PPC does not compete with column liquid chromatography for purification and isolation of compounds from a complex matrix. Instead, the two approaches are complementary and together they enable successful and rapid separation. It is expected that as a result of development of modern forced-flow and multiple-development techniques, PPC will further expand its importance in the isolation and purification of natural and synthetic products.


Further Reading


Radioactivity Detection

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Introduction

Thin-layer chromatography (TLC) is a technique which has been applied to a wide range of chemicals since its introduction in the early 1950s. The only limitation to its use is that a suitable method of detection must be available; however, this limitation is removed when the compounds of interest are radiolabelled. Nevertheless, since the introduction of thin-layer radiochromatography (TLRC), one major drawback in gaining widespread acceptance has been the lack of an easy method to quantify the distribution of radioactivity whilst still maintaining good resolution. The available detection methods have either been very time-consuming (e.g. autoradiography) or labour-intensive (e.g. zonal analysis) or could not match the resolution of the TLC separation itself. Over the years TLRC detectors have evolved and significantly improved, starting with scanners in the 1960s, followed by linear analysers in the 1980s and now the new 1990s generation of bioimaging analysers and InstantImager. The limitation of the scanners and linear analysers is that their resolution is lower than can be achieved by TLC itself. New detector technology such as phosphor imaging will lead to a renaissance in the use of TLRC due to the excellent resolution.

Detection and Measurement

The principal methods for detecting and quantifying radioactivity on TLC plates are autoradiography, zonal analysis (plate scraping followed by liquid scintillation counting) and direct measurement using radiation detectors. The method employed for