Separation sciences

1. Introduction: Fundamentals of Distribution Equilibrium

2. Gas Chromatography (Chapter 2 & 3)

3. Liquid Chromatography (Chapter 4 & 5)

4. Other Analytical Separations (Chapter 6-8)

    a. Planar chromatography

    b. Supercritical fluid chromatography

    c. Electrophoresis

    d. Centrifugation

    e. Field Flow Fractionation

Planar Chromatography

Key Points

Basic concepts

Retention

Efficiency and Resolution

Development: linear, Radial, and multiple development

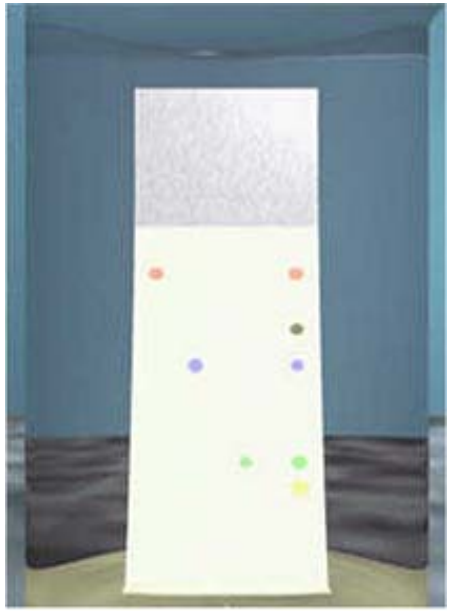
Planar Chromatography

A. Introduction

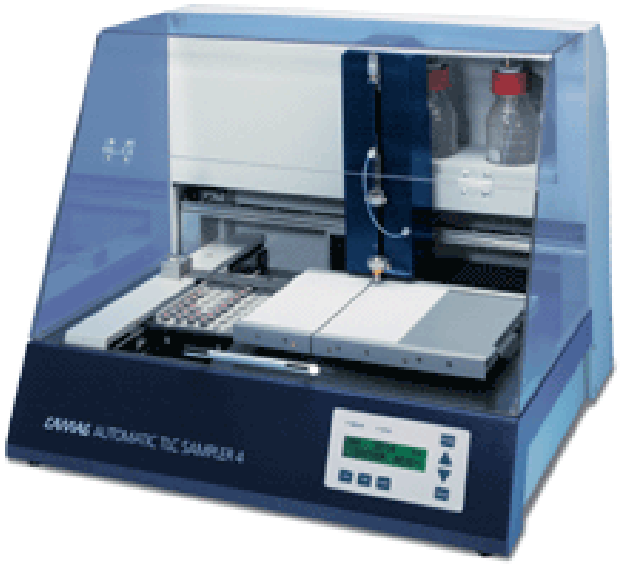
1. Planar chromatography is a chromatographic technique in which the supporting medium is a flat bed or plane rather than a column

2. This was the first type of chromatography developed but is not currently used as much as column chromatographic techniques.

3. Traditionally planar chromatography has been performed by applying a small band a sample to one end of a plate containing a layer of stationary phase. The end of the plate near the point of sample application is then placed in mobile phase and solvent is allowed to migrate up the plate by capillary action. As the solvent travels by the sample, it begins to carry solutes with it. Different solutes will have different interactions with the stationary and mobile phases, making them migrate at different speeds upon the plate.



4. In modern planar chromatography, the same basic approach is used, but the mobile phase may applied by a pump or force other than capillary action. Newer techniques also tend to be more automated than traditional types of planar chromatography in terms of sample application of detection.



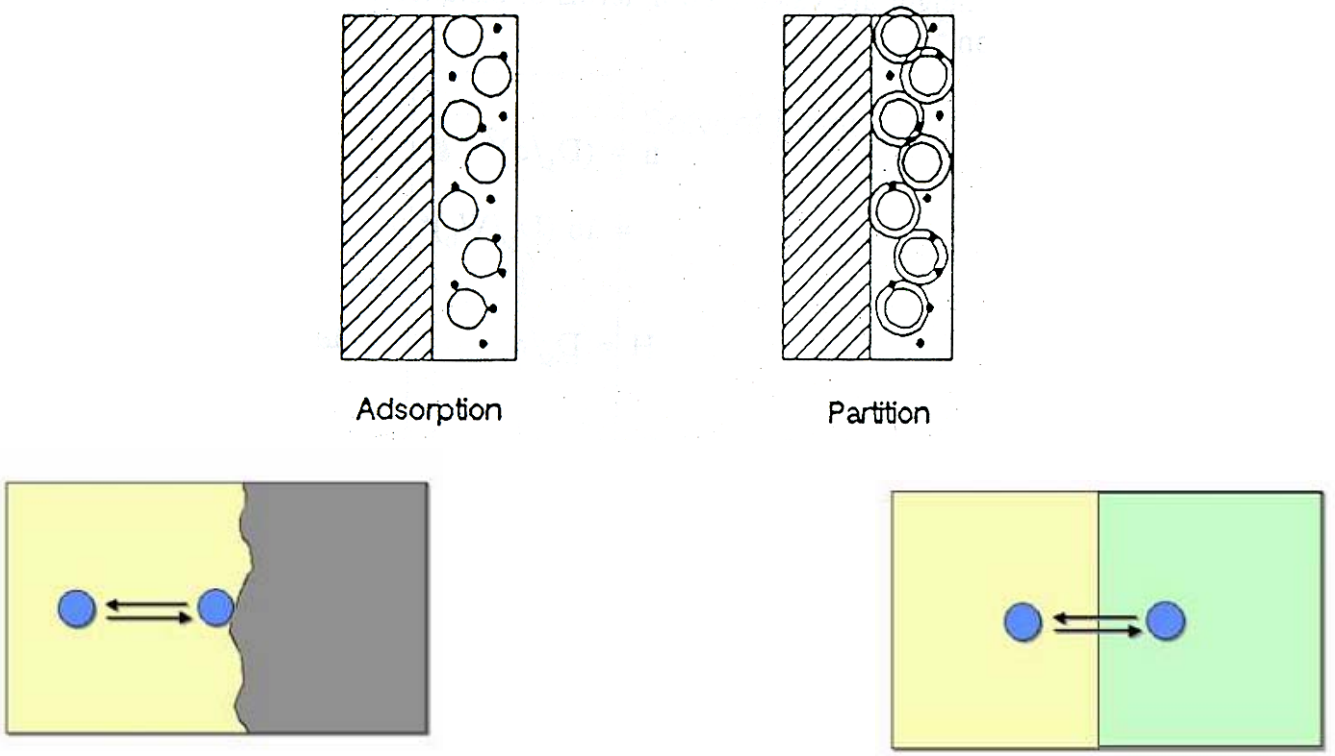
5. Attributes of Planar chromatography

Table 6.1
Attributes of thin-layer chromatography

Attribute	Application
Separation of samples in parallel	•Low-cost analysis and high-throughput screening of samples requiring minimal sample preparation.
Disposable stationary phase	•Analysis of crude samples (minimizing sample preparation requirements) •Analysis of a single or small number of samples when their composition and/or matrix properties are unknown •Analysis of samples containing components that remain sorbed to the separation medium or contain suspended microparticles
Static detection	•Samples requiring postchromatographic treatment for detection •Samples requiring sequential detection techniques (free of time constraints) for identification or confirmation
Storage device	•Separations can be archived •Separations can be evaluated in different locations or at different times •Convenient fraction collection for multimodal column/layer chromatography
Sample integrity	•Total sample occupies the chromatogram not just that portion of the sample that elutes from the column.

B. Theory

1. **As in other types of liquid chromatography, separation in planar chromatography can be based on either adsorption or partitioning.**



2. One difference between column and planar chromatography is the presence of a third phase in the planar system. This third phase is the atmosphere or gaseous vapors located above the surface. In order to obtain reproducible separation, it is necessary to control this phase as well as the stationary and mobile phases.

3. Another difference between planar and column chromatography is that planar chromatography separates based on their different distances of travel in a set amount of time. Column chromatography separates solutes by their different times of travel along a given distance, the length of the column. This difference means that slightly different expressions for retention, efficiency and resolution must be used for planar chromatography.

4. Retention:

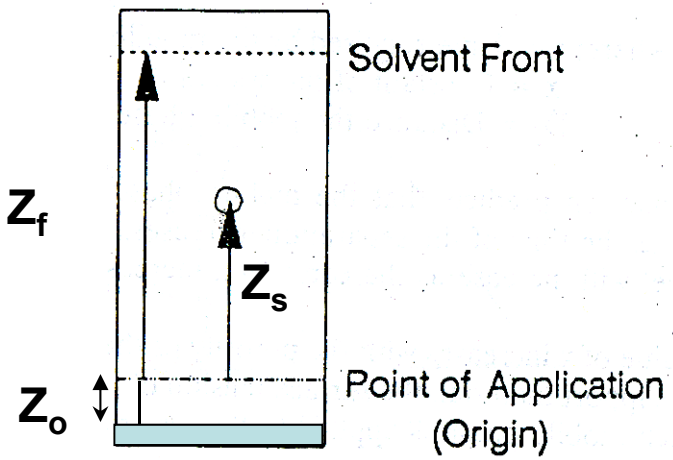
a. The fundamental parameter in TLC is the retardation factor R_f:

$$R_f = Z_s / (Z_f - Z_o)$$

Z_f: Distance traveled by the solvent front from the point of application.

Z_s: Distance traveled by the solute front from the point of application.

Z_o: Distance between the point of application of solvent and solute.



b. The value of R_f is related to the capacity factor (k) of the solute by the following equation:

$$k = (1- R_f)/ R_f$$

c. By using the above equation, planar chromatography can be used to obtain estimates of k for a solute on different stationary phase and mobile phase combinations. This can be useful in screening a number of columns or mobile phase for use in column liquid chromatography.

5. Efficiency

(a) The efficiency of a separation in planar chromatography is described in terms of plates and plate height.

$$N = (Z_s / \sigma)^2$$

$$N = 16*(Z_s / W_b)^2$$

$$H = Z_s /n$$

N: number of theoretical plates; H: plate height.

σ: standard deviation of the solute band (in distance units)

W_b: baseline width of the solute band (in distance units)

b. Note that the efficiency of a planar system is not constant, but depends on the distance that the solute has traveled, or its retention and R_f value.

c. The change in efficiency of a planar chromatography system with distance and the presence of a third phase have made the derivation of exact plate height equations for planar chromatography difficult. These concurrently occur with another complicating factor: the flow rate of mobile phase through a system with capillary flow is not constant with time.

d. For a system with capillary flow, the change in the mobile phase velocity with time is described by the following equation:

$$Z_f = (xt)^{1/2}$$

t : time required by the mobile phase to migrate distance Z_f.
x : the system constant.

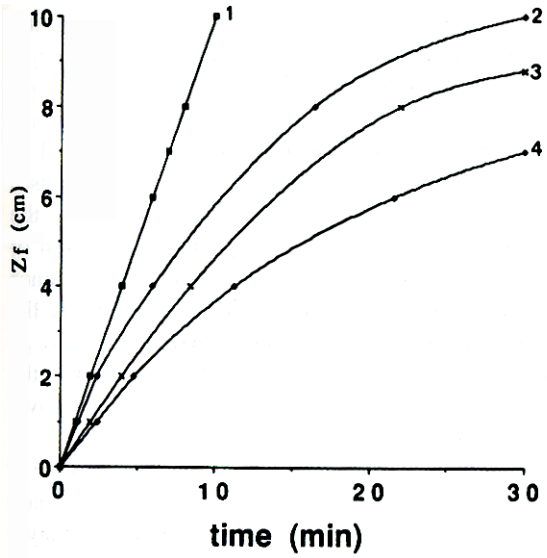


Figure 6.1. Relationship between the solvent-front position and time for a forced flow separation (1) and capillary flow separations with an exposed layer in a saturated chamber (2), a covered layer (sandwich chamber) (3), and an exposed layer in an unsaturated atmosphere (4). (From ref. [33]; ©Research Institute for Medicinal Plants).

e. The plate height equation for a planar system with capillary flow is shown below

$$H_{tot} = a\,[(Z_f^{2/3} - Z_o^{2/3})/(Z_f - Z_o)] + b\,(Z_f + Z_o)$$

$a = 3/2\ A\ d_p\ (d_P/2D)^{1/3}$

$b = 2\ D\ /\ (x\ R_f)$

d_p: size of particles

D: diffusion coefficient of solute

x : system constant

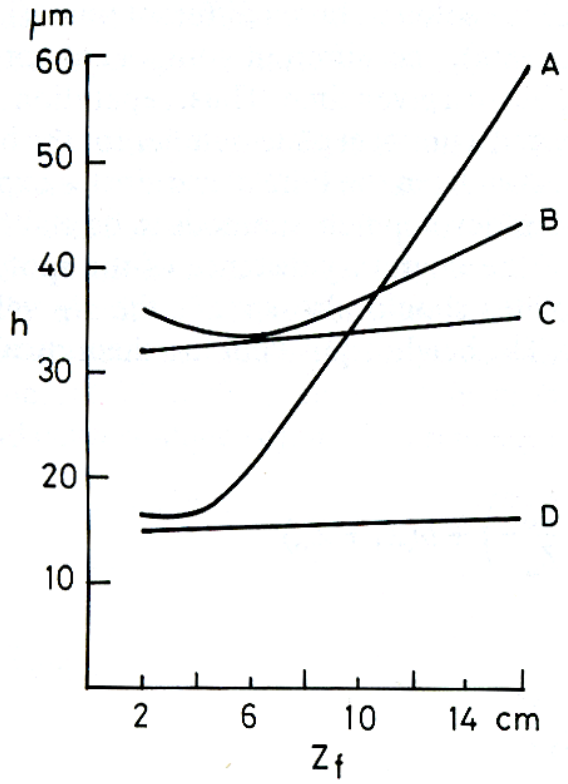
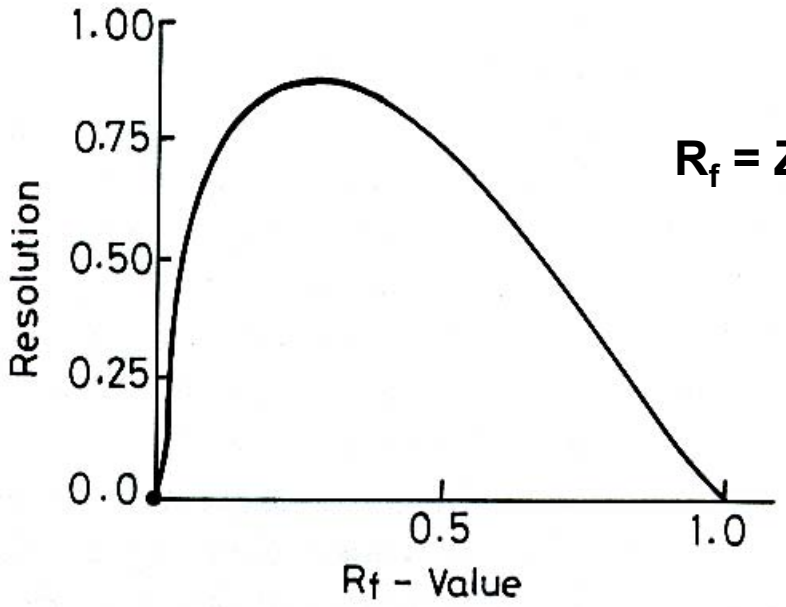


Fig. 8.1 — Variation of theoretical-plate height (h) with solvent-migration distance, Z_f. (A) HPTLC, normal development; (B) conventional TLC, normal development; (C) conventional TLC, over-pressured development; (D) HPTLC, over-pressured development. Reproduced from Ref. 13 by courtesy of Elsevier Scientific Publishing Co.

6. Resolution:

$$R_s = (\alpha\text{-}1)\,[N/(1+k)]^{1/2}[k/(1+k)]$$



$$R_f = Z_s / (Z_f - Z_o)$$

Fig. 8.2 — Variation of resolution in TLC as a function of solute migratory distance (R_F-value). Reproduced from Ref. 13 by courtesy of Elsevier Scientific Publishing Co.

The maximum resolution is achieved at R_f value between 0.2 and 0.5.

C. Types of planar Chromatography

1. There are two main types of planar chromatography
    a. paper chromatography
    b. Thin-layer chromatography (TLC)

2. Paper Chromatography is a special type of planar chromatography in which paper is used as both the support material and stationary phase. Although this method is the historical important, being the first type of chromatography described, it is rarely used in current practice.

3. Thin-layer Chromatography (TLC) is the main type of planar chromatography used at the present. It involves the used of any non-paper materials as the supporting medium.

4. Conventional TLC and high performance TLC (HPTLC): by their separation efficiency.

Table 8.1 — Comparison of the operational parameters of conventional TLC and HPTLC†

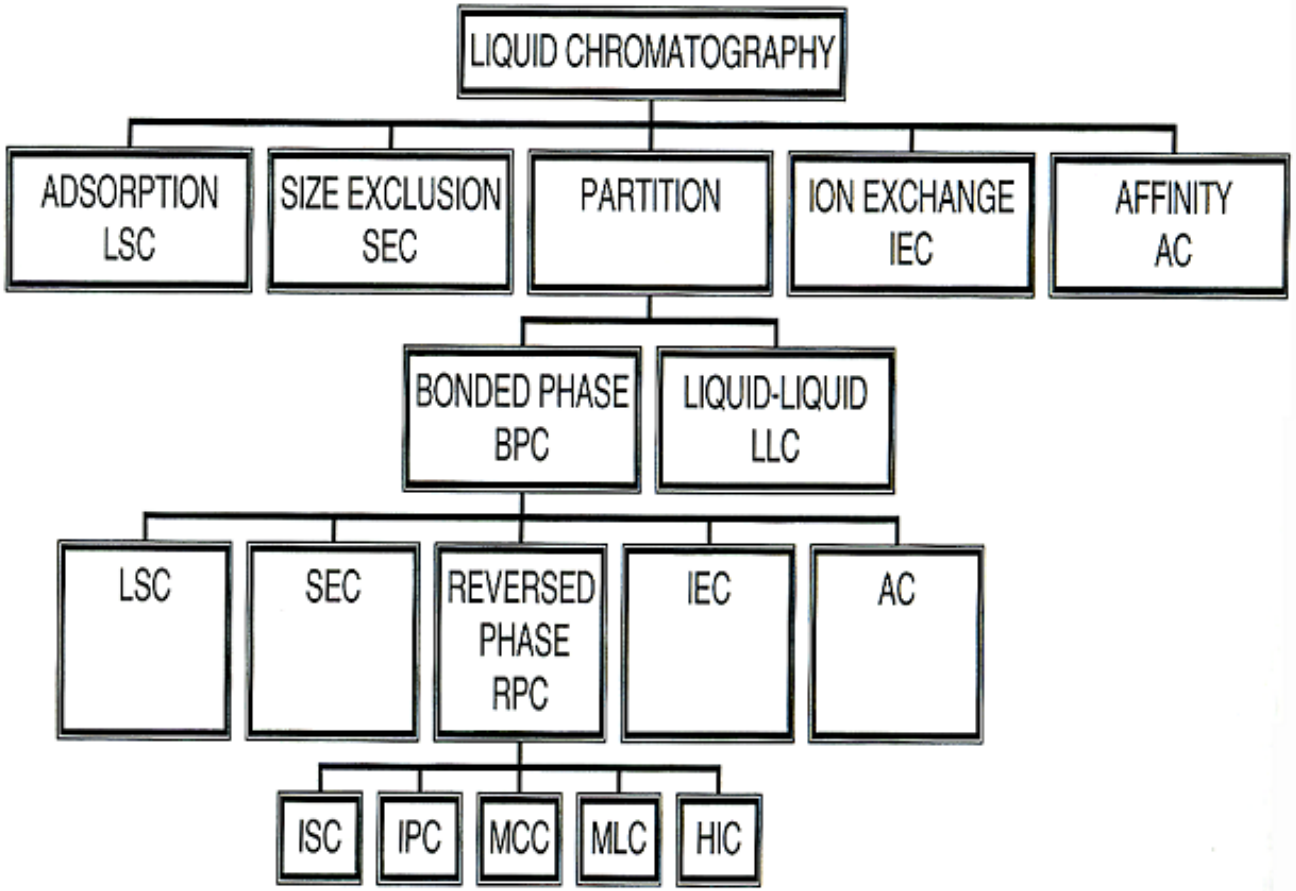
Parameter	Conventional TLC	HPTLC
Plate size	20×20 cm	10×10 cm 10×20 cm
Layer thickness	100–250 μm	200 μm
Particles size		
average	20 μm	5–15 μm
distribution	10–60 μm	narrow
Sample volume	1–5 μl	0.1–0.2 μl
Starting spot diameter	3–6 mm	1.0–1.5 mm
Separated spot diameter	6–15 mm	2–6 mm
Solvent migration distance	10–15 cm	3–6 cm
Time for development	30–200 min	3–20 min
Detection limits		
Absorbance	1–5 ng	0.1–0.5 ng
Fluorescence	0.05–0.1 ng	0.005-0.01 ng
Tracks per plate	10	18 or 36

† Reproduced from Ref. 13 by courtesy of Elsevier Scientific Publishers, Amsterdam.

D. Stationary and mobile phases of TLC

1. The support materials used in TLC are essentially the same as used in column liquid chromatography. These can include materials used n adsorption chromatography (silica and alumina), liquid coated support or bonded-phases for use in partition chromatography (reversed-phase and normal-phase), and support for use in ion-exchange, affinity and size exclusion chromatography.

2. The mobile phase used in TLC are also essentially the same as used in column liquid chromatography. The exactly type used will depend on the stationary phase and type of separation to be performed.



Ion-suppression chromatography
Ion-pair Chromatography
Micellar Liquid Chromatography
Hydrophobic interaction Chromatography (HIC)

E. Elution and Development:

1. Solutes can be eluted in planar chromatography using either isocratic or gradient elution.

2. For gradient elution in TLC

a. Mobile phase gradient

b. stationary phase gradient

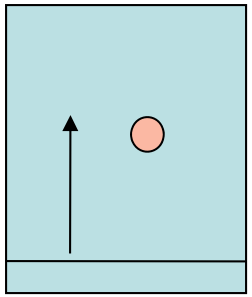
c. Vapor phase gradient

3. Development methods

a. Linear development; b. Radial development; c. Extended development d. Over-pressured or forced-flow development; and e. Multi-dimensional

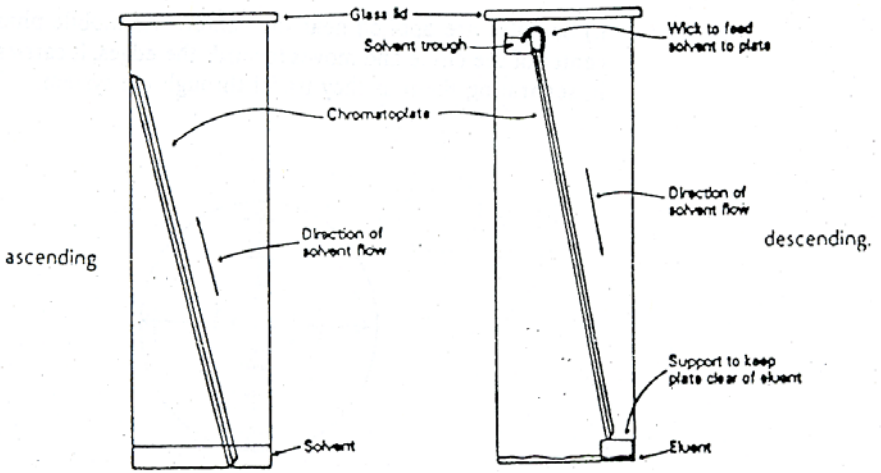
4. Linear development is the most common and familiar of these techniques

a. This technique involves applying solute to a support on a rectangular plate and eluting them with mobile phase flowing in only one direction.

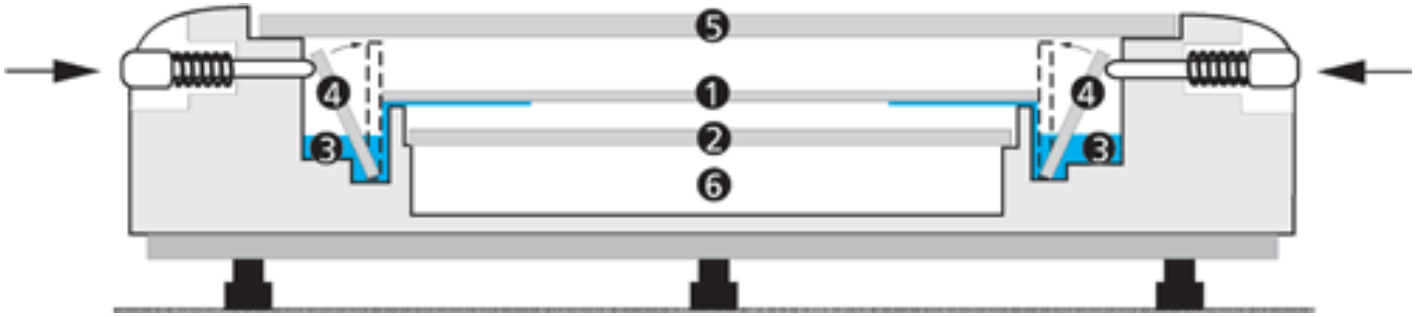


b. Linear development may be performed with the sample and mobile phase applied at the bottom of the support (ascending development), at the top of the support (descending development), or to the edge of a support lying flat (horizontal development).

i. **Ascending and descending development**



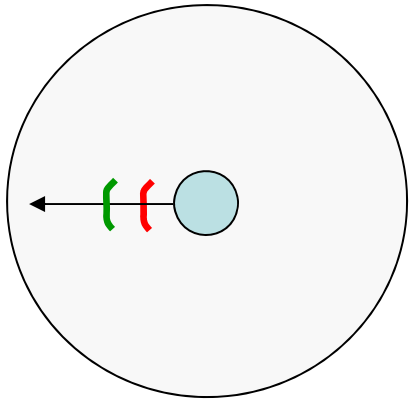
ii. Horizontal development



5. Radial Development is an elution technique also used in planar chromatography. This can be performed in one of two ways: circular development and anti-circular development.

a. In circular development, a flat circular support is used with mobile phase applied to the center.

i. Samples are applied near the center. As mobile phase enters the center of the circle and moves towards the edges, it carries solutes with it, separating them as they travel through the system.



ii. One advantage of using circular development instead of linear development is that resolution is increased (specifically for solutes with high retention factors—low R_f)-- $R_{f(linear)} = R^2_{f(circular)}$

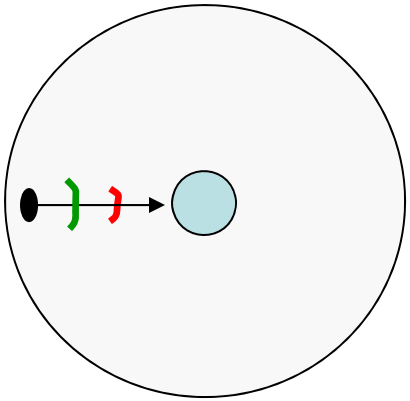
iii. Circular development also avoids the "edge effects" seen in linear development

iv. This system can be used either with capillary flow or forced flow of the mobile. Forced flow can be produced by placing the support disk on a centrifuge and spinning it while mobile phase is applied. This results in a constant flow-rate for the mobile phase, which can be controlled by the rate of rotation of the disk. This technique, also know as centrifugal development, is not only more reproducible and more easily controlled than capillary flow, but also create faster flow-rates and decreases analysis times. The centrifugal development is very useful for preparative separation.

b. In anti-circular development, a flat circular support is used with mobile phase applied at its edges.

i. Samples are applied near the edges and carried toward the center by the mobile phase

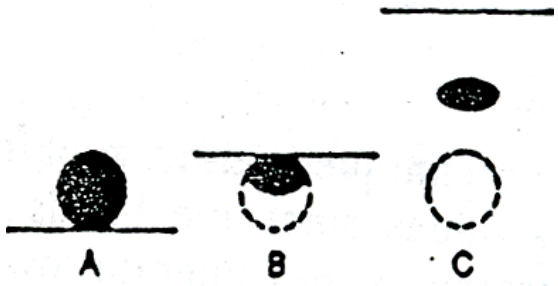
ii. Like circular development, anti-circular development also has advantages of avoiding "edges effects".



6. Extended development (multiple development) is a technique used to improve the separation of closely eluting compounds with low retention--high R_f (R_f > 0.3).

a. Normally such highly retained compounds require very long development times and long plates in order to be resolved when the capillary force is the driving force for mobile phase. However, long development times and distances also make their bands broad, making them more difficult to detect.

b. To minimize broadening of these bands and decrease the time required for the separation, extended development can sometimes be used.



c. Different types of mobile phases can be used in multiple development. It can also be automated by instrument.

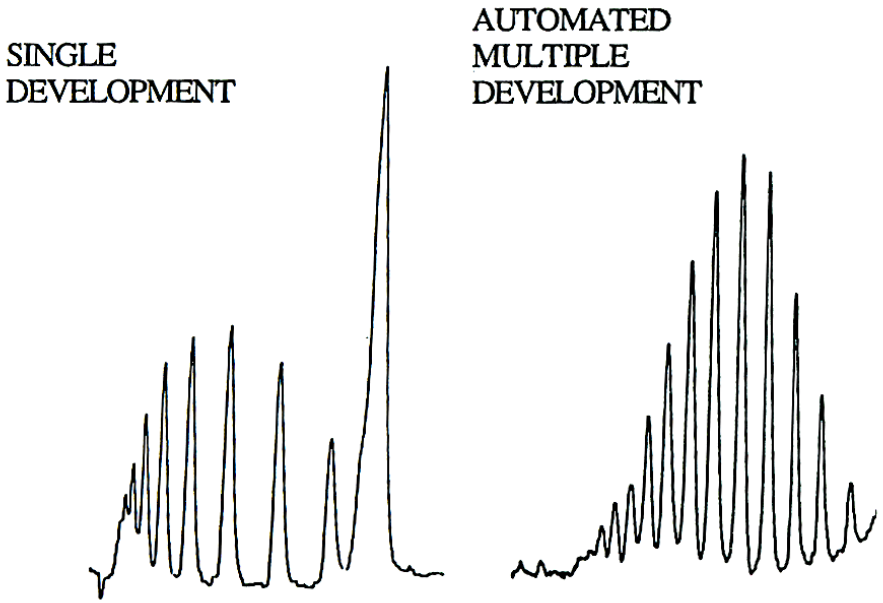
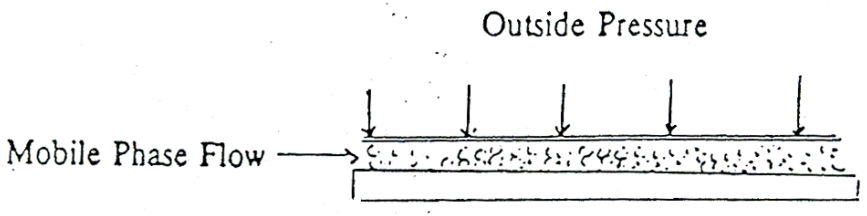


Figure 6.10. Comparison of normal development and incremental multiple development with a decreasing solvent strength gradient (AMD) for the separation of poly(ethylene glycol) 400 as its 3,5-dinitrobenzoate ester. The AMD separation employed a 15- step gradient with methanol, acetonitrile and dichloromethane as solvents. (From ref. [68]. ©Research Institute for Medicinal Plants).

7. Over-pressured, or forced-flow, development is a technique used to improve the resolute, time and efficiency or separation in modern planar chromatography.

Since this is an "enclosed" system, mobile phase may be delivered by a mechanical pump rather than capillary flow. The result is more efficient and faster separations.



8. Multi-dimensional development is a technique that separates solute by combining two or more different elution methods.

a. In the simplest form of this technique, solutes are applied to one corner of a planar support and eluted with a give mobile phase. The support is then rotated 90o and solute are eluted in the second direction by a different mobile phase system. By choosing mobile phase with different strengths and selectivity, the result is a separation of solutes in two directions instead of one.

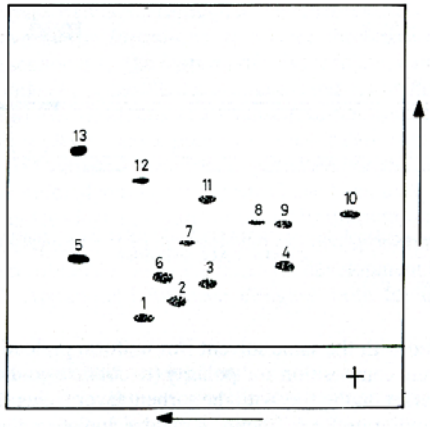


Fig. 8.12 — Illustration of separation of some suphonamides on 2-dimensional development on multilayers using normal and reversed-phase adsorbents. Reproduced from Ref. 19 by courtesy of Whatman Chemical Separations, Inc.

b. Besides using mobile phase, a separation technique may also be used in the second step of a multi-dimensional separation. A common example of this is the use of electrophoresis along with paper chromatography in the separation of amino acids.

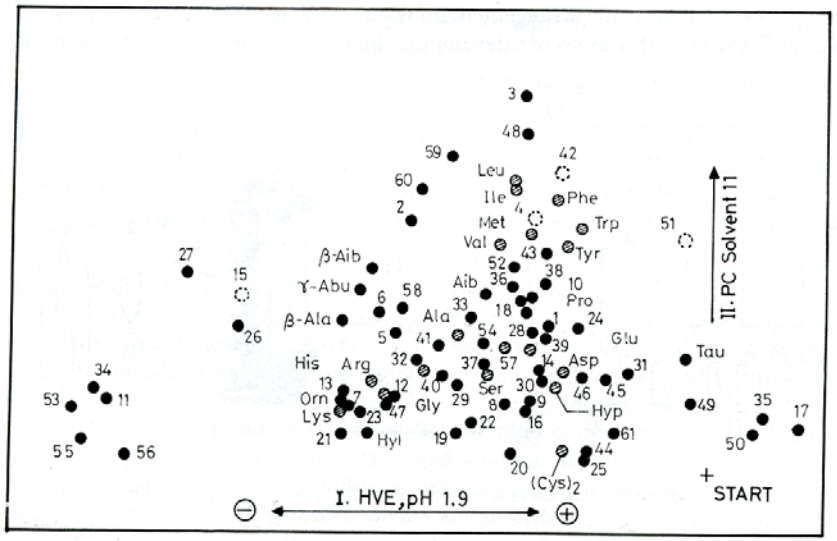


Fig. 8.11 — Illustration of separation of 61 amino acids and peptides by 2-dimensional development using high-voltage electrophoresis at pH 1.9 (horizontal direction) and chromatography on butanol:acetic acid:water (4:1:1) (vertical direction). Reproduced from Ref. 37 by courtesy of Van Nostrand Reinhold Publishing Co.

F. Sample application

1. Sample application is an important feature in obtaining good separations in planar chromatography.

2. Sample application is generally done by placing the solute mixture in a volatile solvent (weak mobile phase) and applying a small amount of the mixture to the support. The solvent is then allowed to evaporate, leaving solute on the plate.

3. In high-performance method, sample spots less than 1 mm width needed. Automatic systems such as robots are typically used.

G. Detection

a. Direct measurement; b. Chemical derivatization.

1. Direct measurement

a. Florescence: emitting light or quenching fluorescence from the substrate.

Green light: manganese-activated zinc silicate, which is stimulated to green fluorescence emission by 254 nm

b. Radioactivity of compounds containing radioisotopes in their structure can also be used to identify the presence of solute bands on a plate. This can be done by using either radioactive detectors, or by exposing film to the surface of the support.

c. Instrumentation detection

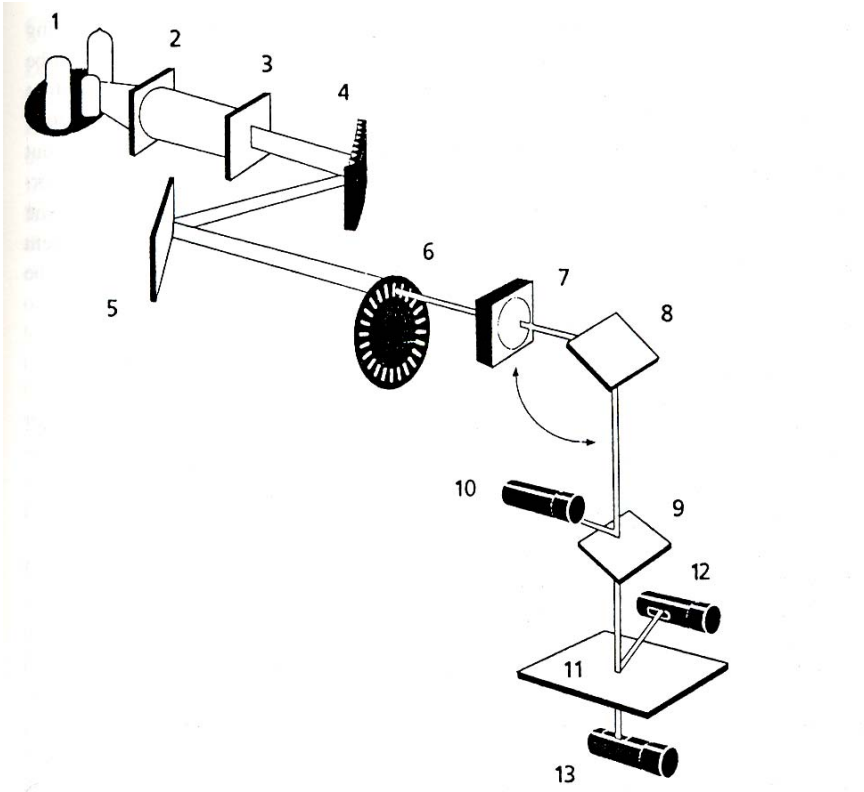


Figure 6.20. Optical arrangement for a slit-scanning densitometer (Camag scanner 3). Identification: 1 = lamp selector; 2 = entrance lens slit; 3 = monochromator entry slit; 4 = grating; 5 = mirror; 6 = slit aperture disk; 7 = lens system; 8 = mirror; 9 = beam splitter; 10 = reference photomultiplier; 11 = TLC plate; 12 = measuring photomultiplier; and 13 = photodiode for transmission measurements. (From ref. [8]; ©Marcel Dekker)

2. Chemical derivatization

a. Iodine adsorption.

b. Oxidation using sulfuric acid

Key Points

Basic concepts

Retention

Efficiency and Resolution

Development: linear, Radial, and multiple development