

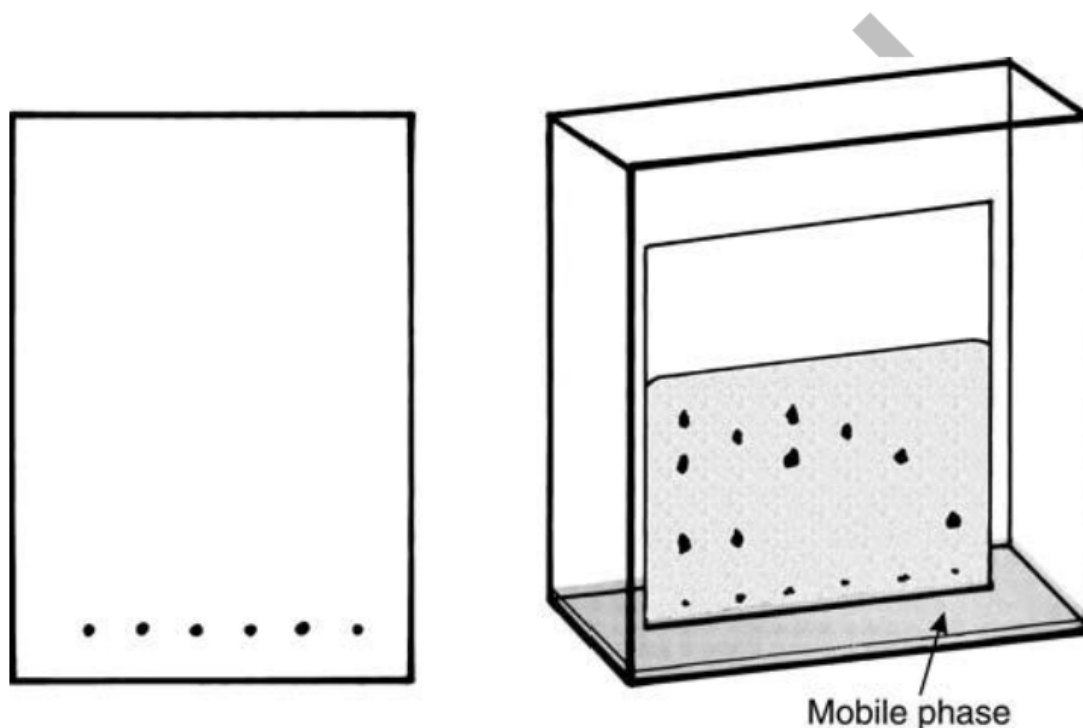
## **Paper and Thin-Layer Chromatography**

Paper chromatography and thin-layer chromatography (TLC) constitute the planar methods mentioned above. Paper chromatography makes use of a sheet of paper having the consistency of filter paper (cellulose) for the stationary phase. Since such paper is hydrophilic, the stationary phase is actually a thin film of water unintentionally adsorbed on the surface of the paper. Thus, paper chromatography represents a form of partition chromatography only. The mobile phase is always a liquid.

With thin-layer chromatography, the stationary phase is a thin layer of material spread across a plastic sheet or glass or metal plate. Such plates or sheets may be either purchased commercially already prepared or prepared in the laboratory.

The thin-layer material can be any of the stationary phases described earlier, and thus TLC can be any of the four types, including adsorption, partition, ion exchange, and size exclusion. Perhaps the most common stationary phase for TLC, however, is silica gel, a highly polar stationary phase for adsorption chromatography, as mentioned earlier. Also common is pure cellulose, the same material for paper chromatography, and here also we would have partition chromatography.

The mobile phase for TLC is always a liquid. The most common method of configuring a paper or thin-layer experiment is the ascending configuration shown below. The mixture to be separated is first spotted (applied as a small spot)



The paper or thin-layer chromatography configuration. Left, the drawing shows the paper or TLC plate with spots applied. Right, the drawing shows the chromatogram in the developing chamber nearing complete development.

within 1 in. of one edge of a 10-in.-square paper sheet or TLC plate. A typical experiment may be an attempt to separate several spots representing different samples and standards on the same sheet or plate. Thus, as many as eight or more spots may be applied on one sheet or plate. So that all spots are aligned parallel to the bottom edge, a light pencil mark can be drawn prior to spotting. The size of the spots must be

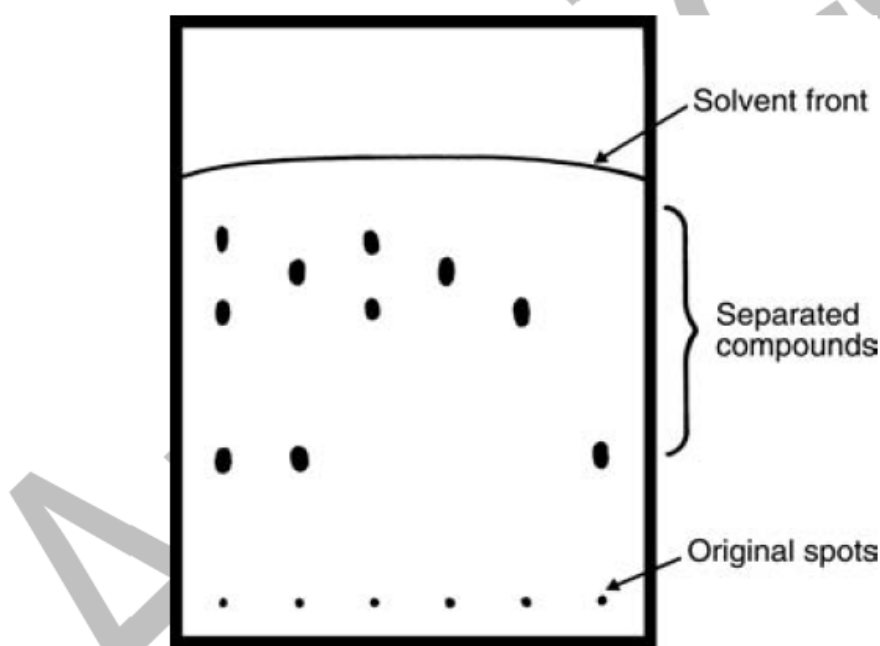
such that the mobile phase will carry the mixture components without streaking.

This means that they must be rather small—they must be applied with a very small diameter capillary tube or micropipet. An injection syringe with a 25- $\mu$ l maximum capacity is usually satisfactory. Following spotting, the sheet or plate is placed spotted edge down in a developing chamber that has the liquid mobile phase in the bottom to a depth lower than the bottom edge of the spots. The spots must not contact the mobile phase. The mobile phase proceeds upward by capillary action (or downward by both capillary action and gravity if in the descending mode) and sweeps the spots along with it.

At this point, chromatography is in progress, and the mixture components will move with the mobile phase at different rates through the stationary phase, and if the mixture components are colored, evidence of the beginning of a separation is visible on the sheet or plate. The end result, if the separation is successful, is a series of spots along a path immediately above the original spot locations, each representing one of the components of the mixture spotted there.

If the mixture components are not colored, any of a number of techniques designed to make the spots visible may be employed. These include iodine staining, in which iodine vapor is allowed to contact the plate. Iodine will absorb on most spots, rendering them visible. Alternatively, a fluorescent substance may be added to the stationary phase prior to the separation (available with commercially prepared plates), such that the spots, viewed under an ultraviolet light, will be visible because they do not fluoresce while the stationary phase surrounding the spots does.

The visual examination of the chromatogram can reveal the identities of the components, especially if standards were spotted on the same paper or plate. Retardation factors (so-called  $R_f$  factors) can also be calculated and used for qualitative analysis. These factors are based on the distance the mobile phase has traveled on the paper (measured from the original spot of the mixture) relative to the distances the components have traveled, each measured from either the center or leading edge of the original spot to the center or leading edge of the migrated spot:



A developed paper or thin-layer chromatogram

$$R_f = \frac{\text{distance mixture component has traveled}}{\text{distance mobile phase has traveled}}$$

These factors, which are fractions less than or equal to 1, are compared to those of standards to reveal the identities of the components. Quantitative analysis is also possible. The spot representing the component of interest can be cut (in the case of paper chromatography) or scraped from the surface (TLC), dissolved, and quantitated by some other technique, such as spectrophotometry. Alternatively, modern scanning densitometers, which utilize the measurement of the absorbance or reflectance of ultraviolet or visible light at the spot location, may be used to measure quantity. Using the TLC concept to prepare pure substances for use in other experiments, such as standards preparation or synthesis experiments, is possible. This is called preparatory TLC and involves a thicker layer of stationary phase so that larger quantities of the mixture can be spotted and a larger quantity of pure component obtained.

Additional details of planar chromatography—methods of descending and radial development, how to prepare TLC plates, tips on how to apply the sample, what to do if the spots are not visible—and the details of preparatory TLC, etc., are beyond our scope.