Thin Layer Chromatography

Chromatographic separations take advantage of the fact that different substances are partitioned differently between two phases, a **mobile phase** and a **stationary phase**. You have already had some experience with **gas chromatography** where the mobile phase is an inert gas, usually helium, and the stationary phase is a high boiling liquid coating absorbed on the surface of a granular solid in a column. In **thin layer chromatography**, or TLC, the mobile phase is a liquid and the stationary phase is a solid absorbent.

Theory of Thin Layer Chromatography

In thin layer chromatography, a solid phase, the **adsorbent**, is coated onto a solid support as a thin layer (about 0.25 mm thick). In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating. Many different solid supports are employed, including thin sheets of glass, plastic, and aluminum. The mixture (A plus B) to be separated is dissolved in a solvent and the resulting solution is spotted onto the thin layer plate near the bottom. A solvent, or mixture of solvents, called the **eluant**, is allowed to flow up the plate by capillary action. At all times, the solid will adsorb a certain fraction of each component of the mixture and the remainder will be in solution. Any one molecule will spend part of the time sitting still on the adsorbent with the remainder moving up the plate with the solvent. A substance that is strongly adsorbed (say, A) will have a greater fraction of its molecules adsorbed at any one time, and thus any one molecule of A will spend more time sitting still and less time moving. In contrast, a weakly adsorbed substance (B) will have a smaller fraction of its molecules adsorbed at any one time, and hence any one molecule of B will spend less time sitting and more time moving. Thus, the more weakly a substance is adsorbed, the farther up the plate it will move. The more strongly a substance is adsorbed, the closer it will stays near the origin.

Several factors determine the efficiency of a chromatographic separation. The adsorbent should show a maximum of selectivity toward the substances being separated so that the differences in rate of elution will be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. Table 1 lists a number of adsorbents in order of adsorptive power.

Table 1. Chromatographic adsorbents. The order in the table is approximate, since it depends upon the substance being adsorbed, and the solvent used for elution.

Most Strongly Adsorbent	Alumina	Al_2O_3
	Charcoal	C
	Florisil	MgO/SiO_2 (anhydrous)
Least Strongly Adsorbent	Silica gel	SiO_2

The eluting solvent should also show a maximum of selectivity in its ability to dissolve or desorb the substances being separated. The fact that one substance is relatively soluble in a solvent can result in its being eluted faster than another substance. However, a more important property of the solvent is its ability to be itself adsorbed on the adsorbent. If the solvent is more strongly adsorbed than the substances being separated, it can take their place on the adsorbent and all the substances will flow together. If the solvent is less strongly adsorbed than any of the components of the mixture, its contribution to different rates of elution will be only through its difference in solvent power toward them. If, however, it is more, strongly adsorbed than some components of the mixture and less strongly than others, it will greatly speed the elution of those substances that it can replace on the absorbent, without speeding the elution of the others.

Table 2 lists a number of common solvents in approximate order of increasing adsorbability, and hence in order of increasing eluting power. The order is only approximate since it depends upon the nature of the adsorbent. Mixtures of solvents can be used, and, since increasing eluting power results mostly from preferential adsorbtion of the solvent, addition of only a little (0.5-2%, by volume) of a more strongly adsorbed solvent will result in a large increase in the eluting power. Because water is among the most strongly adsorbed solvents, the presence of a little water in a solvent can greatly increase its eluting power.

For this reason, solvents to be used in chromatography should be quite dry. The particular combination of adsorbent and eluting solvent that will result in the acceptable separation of a particular mixture can be determined only by trial.

Table 2. Eluting solvents for chromatography

Least Eluting Power (alumina as adsorbent)	Petroleum ether (hexane; pentane)
	Cyclohexane
	Carbon tetrachloride
	Benzene
	DichIoromethane
	Chloroform
	Ether (anhydrous)
	Ethyl acetate (anhydrous)
	Acetone (anhydrous)
	Ethanol
	Methanol
	Water
	Pyridine
Greatest Eluting Power (alumina as adsorbent)	Organic acids

If the substances in the mixture differ greatly in adsorbability, it will be much easier to separate them. Often, when this is so, a succession of solvents of increasing eluting power is used. One substance may be eluted easily while the other stays at the top of the column, and then the other can be eluted with a solvent of greater eluting power. Table 3 indicates an approximate order of adsorbability by functional group.

Table 3. Adsorbability of organic compounds by functional group

Least Strongly Adsorbed	Saturated hydrocarbons; alkyl halides
	Unsaturated hydrocarbons; aIkenyl halides
	Aromatic hydrocarbons; aryl halides
	Polyhalogenated hydrocarbons
	Ethers
	Esters
	Aldehydes and ketones
	Alcohols
Most Strongly Adsorbed	Acids and bases (amines)

Technique of Thin-layer Chromatography

The sample is applied to the layer of adsorbent, near one edge, as a small spot of a solution. After the solvent has evaporated, the adsorbent-coated sheet is propped more or less vertically in a closed container, with the edge to which the spot was applied down. The spot on the thin layer plate must be positioned above the level of the solvent in the container (Figure 24). If it is below the level of the solvent, the spot will be washed off the plate into the developing solvent. The solvent, which is in the bottom of the container, creeps up the layer of adsorbent, passes over the spot, and, as it continues up, effects a separation of the materials in the spot ("develops" the chromatogram). When the solvent front has nearly reached nearly the top of the adsorbent, the thin layer plate is removed from the container (Figure 25).
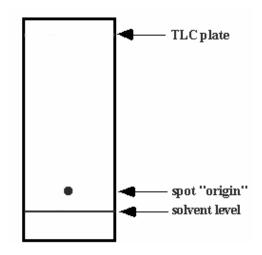
Figure 24. Position of the spot on a thin layer plate.

Since the amount of adsorbent involved is relatively small, and the ratio of adsorbent to sample must be high, the amount of sample must be very small, usually much less than a milligram. For this reason, thin-layer chromatography (TLC) is usually used as an analytical technique rather than a preparative method. With thicker layers (about 2 mm) and large plates with a number of spots or a stripe of sample, it can be used as a preparative method. The separated substances are recovered by scraping the adsorbent off the plate (or cutting out the spots if the supporting material can be cut) and extracting the substance from the adsorbent.
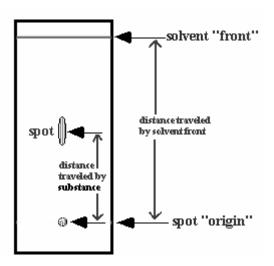

Figure 25. TLC plate showing distances traveled by the spot and the solvent after solvent front nearly reached the top of the adsorbent.

Because the distance traveled by a substance relative to the distance traveled by the solvent front depends upon the molecular structure of the substance, TLC can be used to identify substances as well as to separate them. The relationship between the distance traveled by the solvent front and the substance is usually expressed as the **R_f value**:

$$R_f \text{ value} = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front)}}$$

The R_f values are strongly dependent upon the nature of the adsorbent and solvent. Therefore, experimental R_f values and literature values do not often agree very well. In order to determine whether an unknown substance is the same as a substance of known structure, it is necessary to run the two substances side by side in the same chromatogram, preferably at the same concentration.

Application of the Sample

The sample to be separated is generally applied as a small spot (1 to 2 mm diameters) of solution about 1 cm from the end of the plate opposite the handle. The addition may be made with a micropipet prepared by heating and drawing out a melting point capillary. As small a sample as possible should be used, since this will minimize tailing and overlap of spots; the lower limit is the ability to visualize the spots in the developed chromatogram. If the sample solution is very dilute, make several small applications in the same place, allowing the solvent to evaporate between additions. Do not disturb the adsorbent when you make the spots, since this will result in an uneven flow of the solvent. The starting position can be indicated by making a small mark near the edge of the plate.

Development of thin layer plates

The chamber used for development of the chromatogram (Figure 26) can be as simple as a beaker covered with a watch glass, or a cork-stoppered bottle. The developing solvent (an acceptable solvent or mixture of solvents must be determined by trial) is poured into the container to a depth of a few millimeters. The spotted plate is then placed in the container, spotted end down; the solvent level must be below the spots (see figure below). The solvent will then slowly rise in the adsorbent by capillary action.
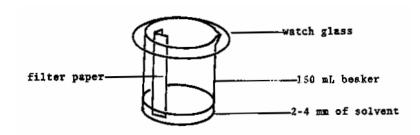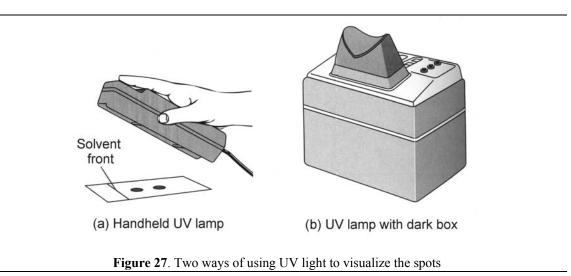

Figure 26. Developing chamber for thin layer chromatogram.

In order to get reproducible results, the atmosphere in the development chamber must be saturated with the solvent. This can be accomplished by sloshing the solvent around in the container before any plates have been added. The atmosphere in the chamber is then kept saturated by keeping the container closed all the time except for the brief moment during which a plate is added or removed.

Visualization

When the solvent front has moved to within about 1 cm of the top end of the adsorbent (after 15 to 45 minutes), the plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are colored, they can be observed directly. If not, they can sometimes be visualized by shining ultraviolet light on the plate (Figure 27) or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapor. Sometimes the spots can be visualized by spraying the plate with a reagent that will react with one or more of the components of the sample.

(a) Handheld UV lamp   (b) UV lamp with dark box

Figure 27. Two ways of using UV light to visualize the spots

General preparation of materials

• The thin layer chromatography plates are commercial pre-prepared ones with a silica gel layer on a glass, plastic, or aluminum backing. Use the wide plates for spotting several compounds on the same plate. This allows for more precise comparison of the behavior of the compounds.

• The samples are spotted on the thin layer plates using fine capillaries drawn from melting point capillaries. You will need to draw several spotters. Your teaching assistant will demonstrate the technique (mystical art?) of drawing capillaries.

• Samples for spotting are prepared by dissolving approximately 0.1 g (the amount on the tip of a spatula) of the compound in less than 0.5 mL of a solvent (ethyl acetate, dichloromethane, or ether work well).

• When spotting samples on the TLC plates, it is a good idea to check if enough sample has been spotted on the plate. Allow the solvent to evaporate and then place the plate under a short wavelength ultraviolet lamp. A purple spot on a background of green should be clearly visible. If the spot is faint or no spot is apparent, more sample will have to be applied to the plate.

• The chromatograms are developed in a 150-mL beaker or jar containing the developing solvent. The beaker is covered with a small watch glass. A wick made from a folded strip of filter paper is used to keep the atmosphere in the beaker saturated with solvent vapor.

• When the plates are removed from the developing solvent, the position of the solvent front is marked, and the solvent is allowed to evaporate. The positions of the spots are determined by placing the plates under a short wavelength ultraviolet lamp. The silica gel is mixed with an inorganic phosphor which fluoresces green in the UV light. Where there are compounds on the plates, the fluorescence is quenched and a dark purple spot appears.