# CHEM 344 Thin Layer Chromatography

Thin layer chromatography (TLC) is a simple analytical technique used for the separation and identification of compounds from mixtures. The TLC technique uses the same principle as extraction to accomplish the separation of compounds: that is, the partitioning of compounds between two phases based on differences in physical properties of the compounds. In the case of TLC, one phase is a mobile liquid solvent phase and the other phase is a stationary solid phase with a high surface area. The **stationary phase** normally consists of a thin layer of finely divided adsorbent, typically silica (SiO_2) or alumina (Al_2O_3) powder, on a supporting material of glass or metal foil. The **mobile phase** is an organic solvent or mixture of solvents.

TLC is routinely used in organic chemistry to monitor the progress of a reaction by observing the disappearance of starting materials and the appearance of products (and byproducts) over time. A solution of the reaction mixture is applied to the edge of the TLC plate as a small spot. The plate is propped vertically in a closed container (developing chamber), with the edge to which the spot was applied resting on the bottom of the chamber in a shallow pool of solvent. The solvent travels up the plate by capillary action, passes over the sample spot and moves the compounds in the mixture along the plate at different rates, resulting in separation of the compounds. This process is termed elution.

In the example shown below (Figure 1), the reaction mixture $A + B \rightarrow C$ was sampled at 4 different time periods (10, 20, 40, and 60 min, shown on base of plate). Pure compounds A, B, and C were also spotted for comparison. Development of the plate reveals that compounds A and B react to yield C within 60 min.
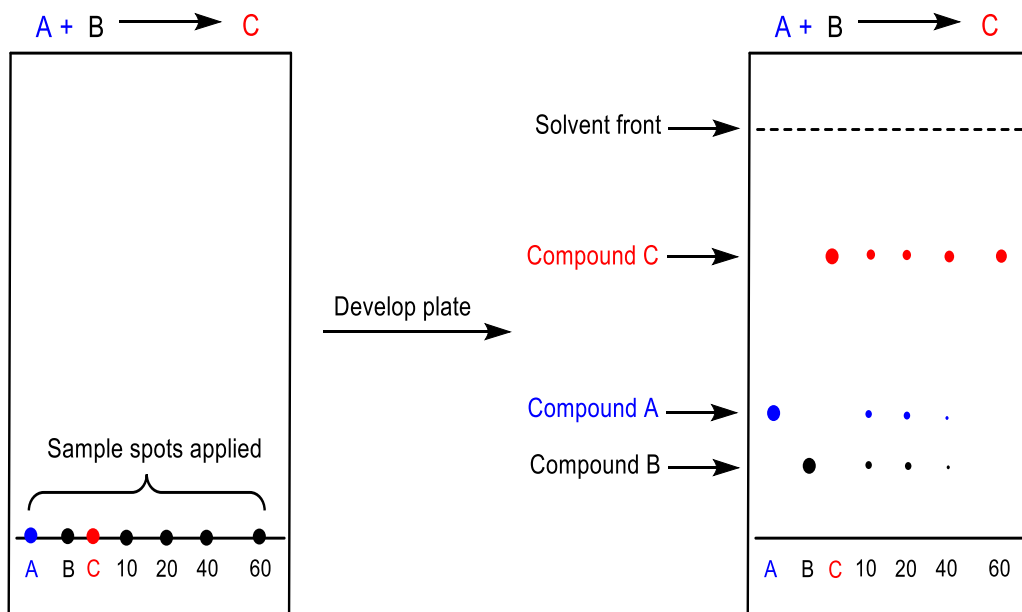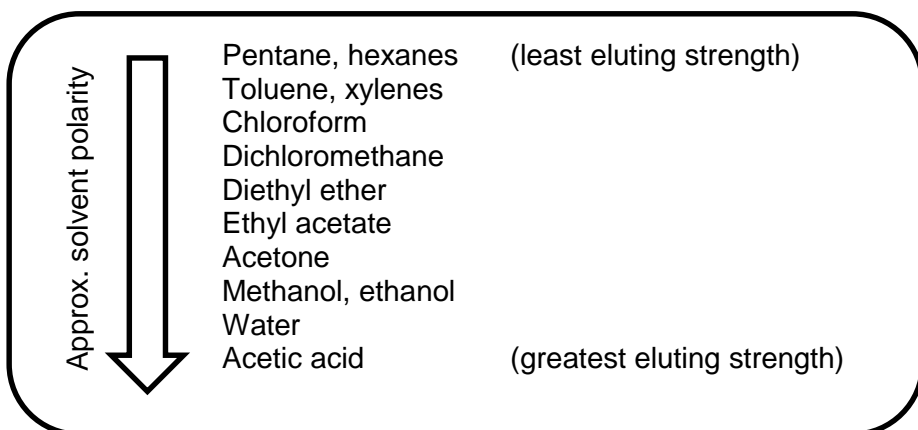

Figure 1. TLC plate used to monitor the reaction $A + B \rightarrow C$.

An equilibrium is established between the molecules of each compound (A, B, or C) adsorbed onto the surface of the plate and the molecules of the compound which are in solution. Each component of the mixture will differ in solubility and in the strength of its adsorption to the plate, and thus as the mobile phase flows over the surface of the plate each component is carried up the plate to a differing extent. This forms the basis of separation and identification of the components of the A + B → C reaction mixture.

When the solvent front reaches near the top of the plate, the plate is removed from the developing chamber, the solvent front is marked with a pencil, the plate allowed to dry, and the separated components of the reaction mixture (the "spots") are visualized. Visualization is straightforward if the compounds are highly colored. Typically, however, the separated organic compounds are colorless or only weakly colored and so a UV lamp is required to visualize the plates. (The TLC plate is coated with an inert fluorescent dye which glows under UV radiation *except* where an organic compound is on the plate). The overall procedure is referred to as "developing" the TLC plate.

The solvent used as the mobile phase should be able to dissolve all of the compounds to be separated. The solubility of different compounds in the solvent plays an important role in how rapidly they move up the TLC plate. A more important property of the solvent is its ability to be adsorbed onto the plate. To the extent that the solvent has affinity for the adsorbent (typically SiO_2), it can displace the compounds in the reaction mixture thereby "pushing" them up the plate. If the solvent is too strongly adsorbed, it can fully displace all compounds causing them to move up the plate together near the solvent front, resulting in minimal (or no) separation of the mixture. If the solvent is too weakly adsorbed, its solvating power alone may be insufficient to move any compounds along the plate fast enough to effect separation. Ideally, the affinity of the solvent for the adsorbent should be similar to that of the compounds being separated, causing different compounds to move at different rates and resulting in adequate separation of the mixture.

Approx. solvent polarity →		
Pentane, hexanes	(least eluting strength)
Toluene, xylenes	
Chloroform	
Dichloromethane	
Diethyl ether	
Ethyl acetate	
Acetone	
Methanol, ethanol	
Water	
Acetic acid	(greatest eluting strength)

Figure 2. List of common organic solvents in order of increasing eluting strength.

Because the eluting strength of a solvent is primarily related to how strongly it adsorbs onto the adsorbent and because typical adsorbents are highly polar, eluting strength increases with solvent polarity. In practice, mixtures of solvents are commonly used to achieve optimum separations by TLC. When using mixtures of solvents, addition of a minor amount of a polar solvent can result in a large increase in the eluting power of the solvent mixture.

Silica gel (SiO_2) consists of a three-dimensional network of Si-O bonds, with Si-O-H groups on the surface. A silica gel TLC plate is essentially a thin layer of very finely ground pure sand adhered to a metal or glass support (Figure 3).
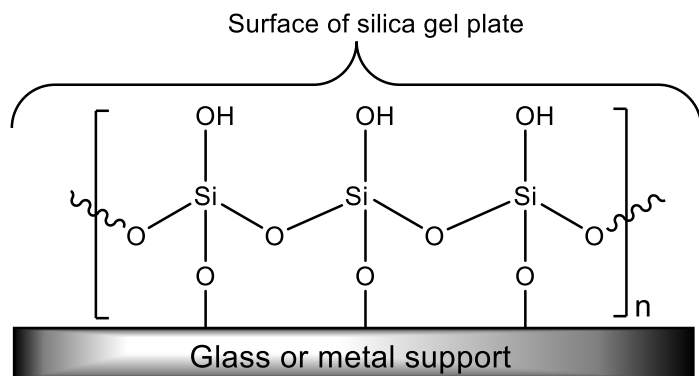
Figure 3. Surface of a silica gel TLC plate.

Molecules featuring a significant dipole interact strongly with the polar Si-OH groups at the surface of the SiO_2 plate and thus will adsorb onto the fine particles of the adsorbent. In contrast, molecules with a weak dipole (or no dipole) interact less strongly with the surface of the polar Si-OH network on the surface of the plate. Molecules with a weak dipole generally move through along the Si-OH network more rapidly than those with a significant dipole and thus appear higher on the plate once it is developed (Figure 4).

It is possible to make some approximations about the relative rate of elution of different compounds with a given solvent (or mixture of solvents) on a specific adsorbent, however the specific combination that results in the successful separation of a specific mixture of compounds can be determined only by experimentation. The process begins by consideration of the structures of the compounds to be separated and their relative affinity for the stationary phase. Figure 4 indicates an approximate order of affinity of organic compounds for SiO_2 sorted by functional group. The strength with which an organic compound binds to the TLC plate depends primarily upon the extent of the dipole-dipole interactions between the molecule and the surface of the plate. Other attractive forces between the adsorbent and the organic compounds (such as hydrogen bonding, dipole-induced-dipole, and van der Waals forces) also play a role in the overall strength of the molecule-surface interaction.
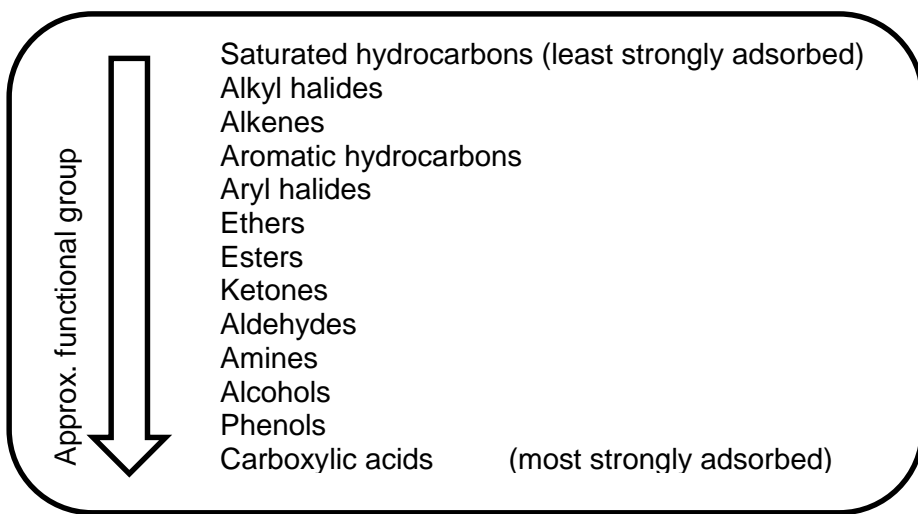

Figure 4. Affinity of organic compounds for SiO_2 by functional group.

The distance traveled by a compound relative to the distance traveled by the solvent front depends upon the structure of the molecule, and thus TLC can be used to identify substances as well as to separate them. But how is the extent of interaction between the molecule and the plate surface quantified?

The relationship between the distance traveled by the compound(s) and the distance traveled by the solvent front and is expressed as a decimal, termed the **R_f value (retention factor)**. The stronger a compound is bound to the adsorbent, the slower it moves up the TLC plate and thus the lower its R_f value.

In the example below (Figure 5), compound C has traveled furthest up the TLC plate because it is the least strongly adsorbed compound of the mixture and thus has the highest R_f value (0.76). Likewise, B has the lowest R_f value (0.29) and is thus the most strongly adsorbed compound. Figure 2 shows how to calculate R_f values for the components of the reaction mixture A + B → C.

$$R_f = \frac{Distance\ Traveled\ by\ Compound\ (mm)}{Distance\ from\ origin\ to\ Solvent\ front\ (mm)}$$
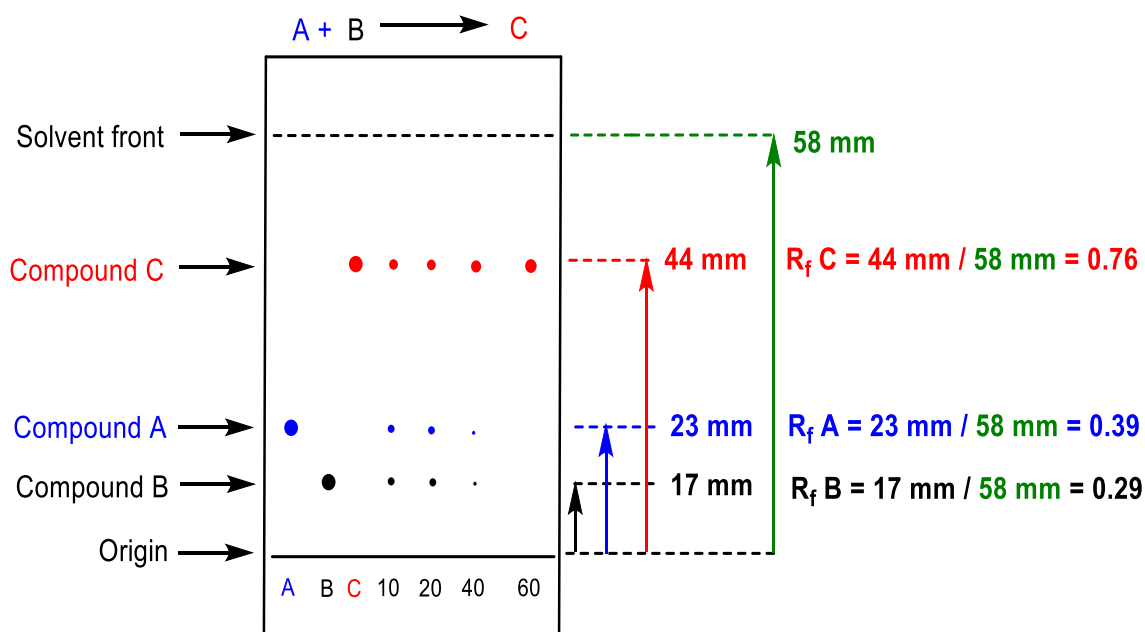
Figure 5. Calculation of R_f values for compounds A, B, and C.

Experimental R_f values are strongly dependent upon the nature of the plate surface and the solvent system, and thus experimental R_f values obtained from different TLC runs are not always in agreement. In order to determine whether an unknown compound is identical to a compound of known structure, it is necessary to run samples of the two compounds side-by-side on the same TLC plate, preferably at the same concentration. This concept is illustrated in Figure 1 and 5, where A, B, and C are spotted on the TLC plate as pure compounds in order to compare their R_f values with those obtained from the reaction mixture.