Thin Layer Chromatography.

Thin layer chromatography, or **TLC**, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analyzed by TLC - and it takes little time for an analysis (about 5-10 minutes).

TLC consists of three steps - spotting, development, and visualization. Photographs of each step are shown on the course website. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipet to transfer a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates and leaves behind a small spot of the material.

Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate.

Visualization of colored compounds is simple – the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. It is good practice to use at least two visualization techniques in case a compound does not show up with one particular method.

The Rf value is used to quantify the movement of the materials along the plate. Rf is equal to the distance traveled by the substance divided by the distance traveled by the solvent. It's value is always between zero and one. A TLC analysis might be summarized something like, "Using a silica gel plate and ethyl acetate as the development solvent,

unknown mixture X showed three spots having Rf's of 0.12, 0.25, and 0.87". Comparing these Rf's with the Rf's of known compounds might enable a tentative identification to be made. <u>Note that observing three spots means only that there are at least three components in the mixture</u>. Some components may have such similar polarities that they appear under one spot after development.
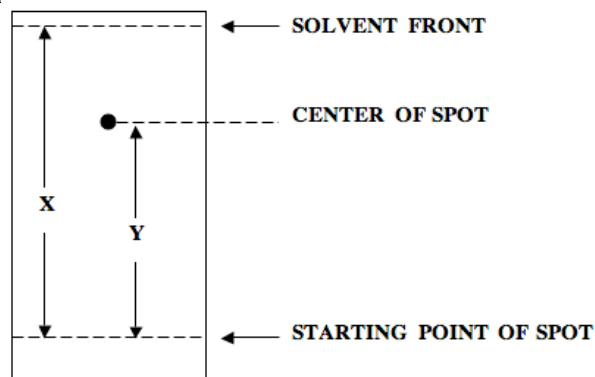

$$R_f = Y/X \text{ (always} \le 1)$$

If a development solvent of too high a polarity is used, all components in the mixture will move along with the solvent and no separation will be observed (Rf's will be too large). If the solvent is of too low a polarity the components will not move enough, and again separation will not occur (Rf's will be too small). In practice, different solvents or mixtures of solvents are tried until a good separation is observed. Typically an effective solvent is one that gives Rf's in the range of 0.3 - 0.7.
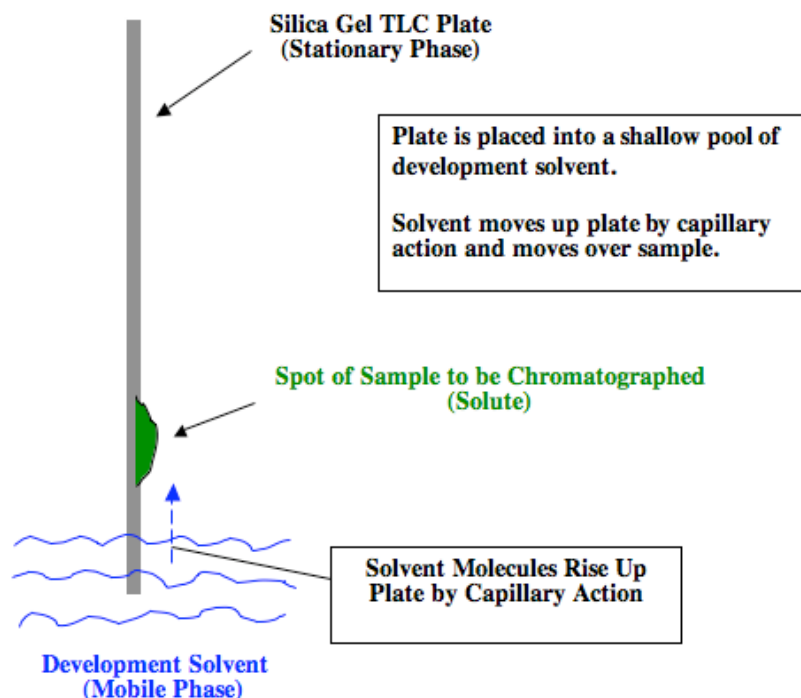
Note that the spotting solvent is simply used as a vehicle to transfer the material to be analyzed to the TLC plate. Once the transfer is made the spotting solvent evaporates. It has no effect on the separation. It is the development solvent that effects the separation.

<u>What's going on at the molecular level during development</u>? There are three components in TLC: (1) the TLC plate (stationary phase), the development solvent (mobile phase), and the sample to be analyzed (solute). In our experiment the TLC plate consists of a thin plastic sheet covered with a thin layer of silica gel, a portion of the structure of which is shown below.
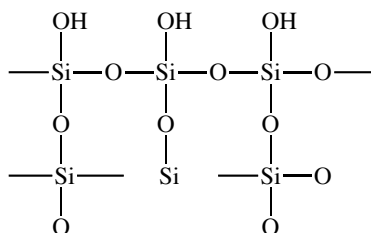

Silica gel consists of a three-dimensional network of thousands of alternating silicon and oxygen bonds, with O-H groups on the outside surface. Silica gel is simply very finely ground very pure sand. It should be noted that silica gel is highly polar and is capable of hydrogen bonding.

Consider the side-on view of the development of a TLC plate below. As the solvent travels up the plate, over the spot, an equilibrium is set up, as development solvent competes with the TLC plate for the solute. The silica gel binds to the solute and the development solvent tries to dissolve it away, carrying the solute(s) along as the solvent travels up the plate.

Silica Gel TLC Plate
(Stationary Phase)

Plate is placed into a shallow pool of development solvent.

Solvent moves up plate by capillary action and moves over sample.

Spot of Sample to be Chromatographed
(Solute)

Solvent Molecules Rise Up Plate by Capillary Action
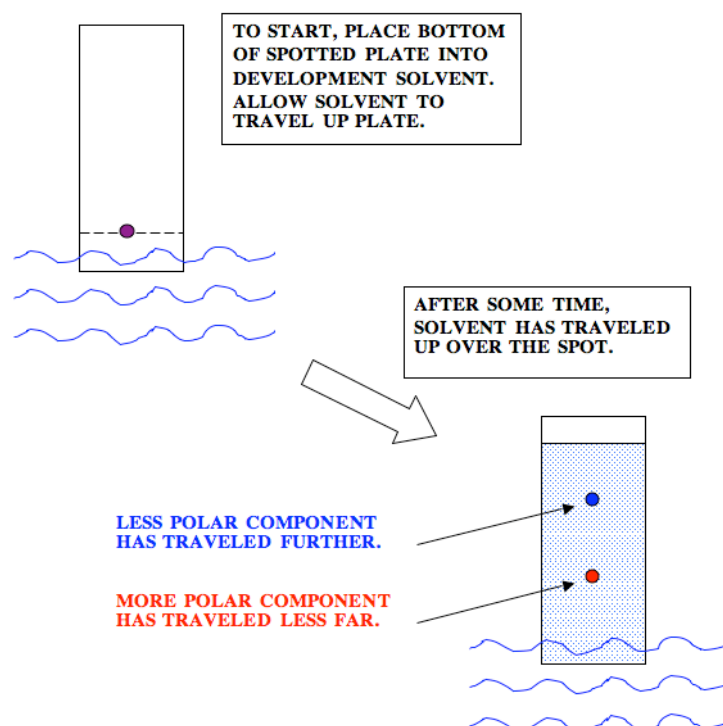
Development Solvent
(Mobile Phase)

A balance of intermolecular forces determines the position of equilibrium and thus the ability of the solvent to move the solute up the plate. In other words, would the spot prefer to be stuck on the plate or would it prefer to move along with solvent.
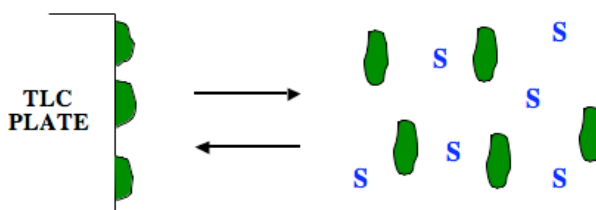
TLC PLATE

The balance depends upon (1) the polarity of the TLC plate (constant and high), (2) the polarity of the development solvent (can be varied by using different solvents), and (3) the polarity of the compounds in the spot (this varies depending upon what compounds are in the spot). For example, if a sample consists of two components, one more polar than the other, the more polar will tend to stick more tightly to the plate and the less polar will tend to move along more freely with the solvent. Using a more polar development solvent would cause both to move along further. If the approximate structures of the solutes are known, it is possible to make an educated guess as to what solvent or mixture of solvents to use. In practice though, for a given mixture of compounds to be analyzed, a solvent or mixture of solvents is chosen by trial and error to give the best separation. (A

caveat: the polarity argument is helpful in understanding the principles of TLC. Because most compounds have some polarity the argument works well. For compounds having very low polarity however, a lower-polarity solvent may be more effective in moving the solute up the plate.)

To illustrate a TLC experiment, consider the following example of the analysis of a two-component mixture.

TO START, PLACE BOTTOM OF SPOTTED PLATE INTO DEVELOPMENT SOLVENT. ALLOW SOLVENT TO TRAVEL UP PLATE.

AFTER SOME TIME, SOLVENT HAS TRAVELED UP OVER THE SPOT.

LESS POLAR COMPONENT HAS TRAVELED FURTHER.

MORE POLAR COMPONENT HAS TRAVELED LESS FAR.

The polarity of molecules, solutes and solvents alike, is ordered as follows, from least to most polar:

Alkanes (least polar), alkyl halides, alkenes, aromatic hydrocarbons, ethers, esters, ketones, aldehydes, amines, alcohols, and carboxylic acids (most polar). Note however that many molecules contain multiple functional groups and that the overall polarity would be determined by all of the groups.