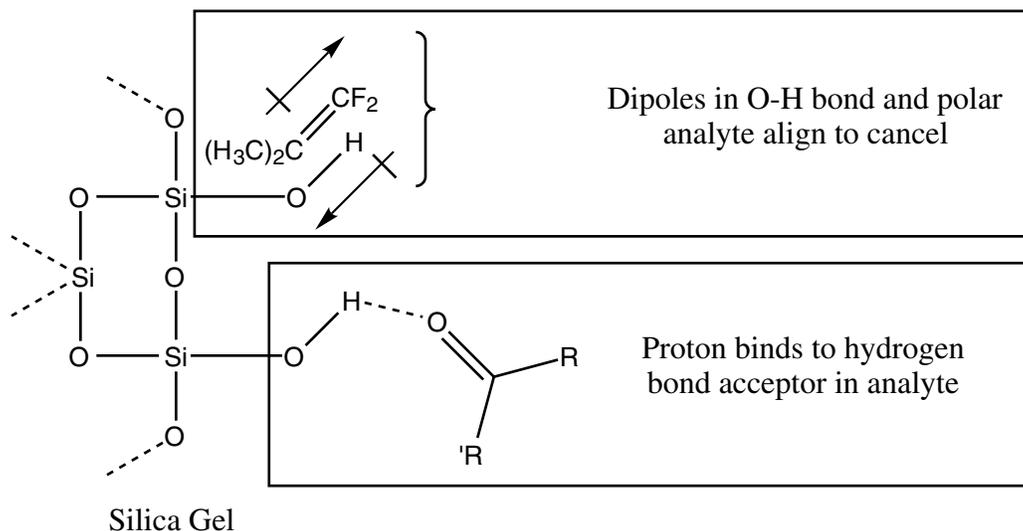# Thin Layer Chromatography

Chemistry 136

Thin layer chromatography (TLC) is among the most useful tools for following the progress of organic chemical reactions and for assaying the purity of organic compounds. TLC requires only a few ng (yes that's right nano grams!) of sample for a successful analysis and can be accomplished in a matter of minutes. Like all chromatographic methods, TLC takes advantage of the different affinity of the analyte with the mobile and stationary phases to achieve separation of complex mixtures of organic molecules.

Theory of Chromatography

Stationary Phase

Silica gel, the most commonly used stationary phase, has the empirical formula SiO_2. However, at the surface of the silica gel particles, the dangling oxygen atoms are bound to protons. The presence of these hydroxyl groups renders the surface of silica gel highly polar. Thus, polar functionality in the organic analyte interacts strongly with the surface of the gel particle and nonpolar functionality interact only weakly. Polar functionality in the analyte molecules can bind to the silica gel in two ways: through hydrogen bonds and through dipole-dipole interactions. The total strength of the interaction is a sum of these two components. It should be noted that the shape of the organic analyte is also a factor in predicting the strength of its interaction with silica gel. Thus, an analyte that displays multiple polar groups in position to interact with the surface of the stationary phase with interact more strongly than an analyte that displays the same polar functionality in a way that does not permit multidentate binding.

Modes of Interaction of Analyte with Silica Gel

Dipoles in O-H bond and polar analyte align to cancel

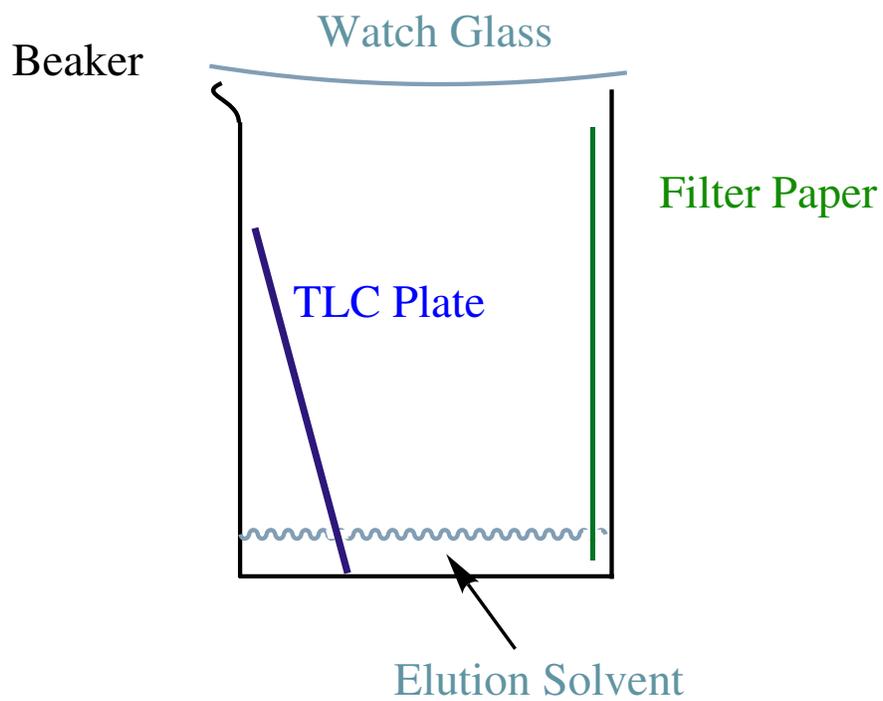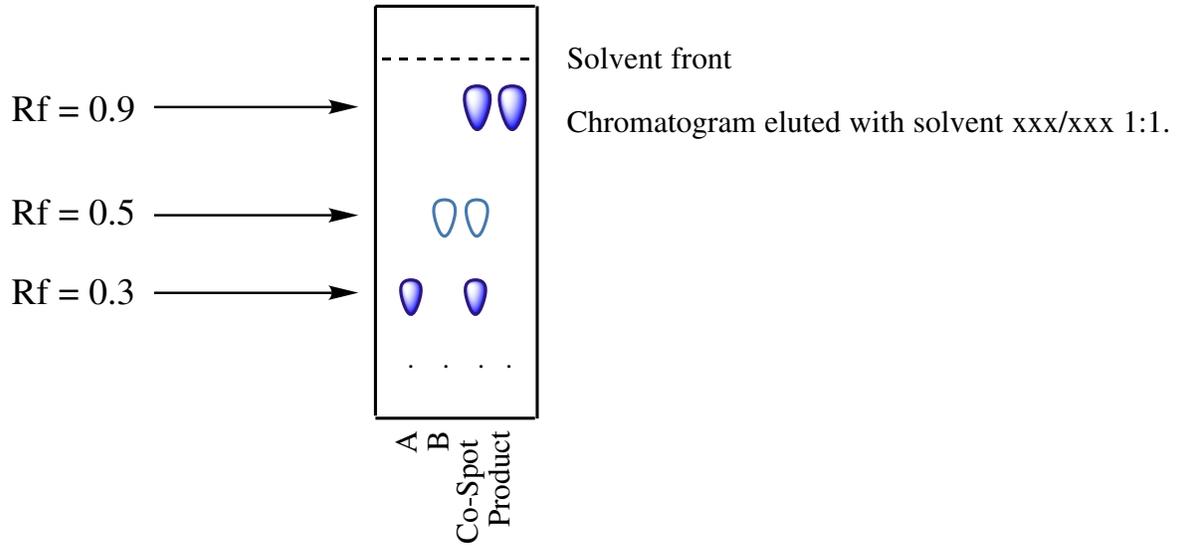Proton binds to hydrogen bond acceptor in analyte

Silica Gel

Mobile Phase

For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves past the surface of the silica gel it transports the analyte past the particles of the stationary phase. However, the analyte molecules are only free to move with the solvent if they are not bound to the surface of the silica gel. Thus, the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte. The ability of an analyte to bind to the surface of the silica gel in the presence of a particular solvent or mixture of solvents can be viewed as a the sum of two competitive interactions. First, polar groups in the solvent can compete with the analyte for binding sites on the surface of the silica gel. Therefore, if a highly polar solvent is used, it will interact strongly with the surface of the silica gel and will leave few sites on the stationary phase free to bind with the analyte. The analyte will, therefore, move quickly past the stationary phase. Similarly, polar groups in the solvent can interact strongly with polar functionality in the analyte and prevent interaction of the analyte with the surface of the silica gel. This effect also leads to rapid movement of the analyte past the stationary phase. The polarity of a solvent to be used for chromatography can be evaluated by examining the dielectric constant (ε) and dipole moment (δ) of the solvent. The larger these two numbers, the more polar is the solvent. In addition, the hydrogen bonding ability of the solvent must also be considered. For example methanol is a strong hydrogen bond donor and will severely inhibit the ability of all but the most polar analytes to bind the surface of the silica gel.

The TLC Experiment

The first step in conduction a TLC experiment is to select the elution solvent. For most organic molecules, a good starting point is 2 parts ethyl acetate to 3 parts hexanes. Place about 10 mL of the elution solvent in a 100 mL beaker covered with a watch glass. To ensure that the atmosphere in the elution chamber is saturated with solvent vapor, place a piece of filter paper, torn into a square, along the inside wall of the beaker. Be sure that the bottom of the filter paper touches the solvent. Using a pencil, draw a line on the TLC plate about 5 mm from the bottom. Cross the line in three places with short pencil lines. These three intersections are the locations onto which you will place the sample. Prepare a solution of you sample in the least polar solvent in which it is soluble. About 1 mg (a speck) of sample dissolved in two to three drops of solvent is all that is required. The sample is introduced onto the TLC plate using a micro capillary. Dip the end of the micro capillary into the sample solution. A small volume of the solution will flow into the micro capillary. Now you can spot the capillary onto the pencil lines on your TLC plate. Be sure that the spots on the TLC plate are no more than 3 mm in diameter. Let the spotting solvent evaporate for a few seconds and then place the TLC plate in the elution chamber with the sample spots at the bottom. Note that the sample spots should be above the level of the elution solvent. If this is not the case, use a pipette to remove a small amount of the elution solvent from the chamber. Now let the sample elute to a point where the solvent front is about 5 mm from the top of the TLC plate. To visualize the spots on you TLC plate you will use UV light, iodine or a series of chemical stains. You may need to adjust the polarity of the solvent if the retention factor (Rf) of you analyte is too large or too small. The Rf is calculated by dividing the distance traveled by the analyte by the distance traveled by the solvent. The ideal solvent gives the analyte an Rf of 0.3.

Example TLC Plate

Solvent front

Rf = 0.9

Chromatogram eluted with solvent xxx/xxx 1:1.

Rf = 0.5

Rf = 0.3

A
B
Co-Spot
Product

Watch Glass

Beaker

Filter Paper

TLC Plate

Elution Solvent

C. Eluotropic Series: Relative Solvent Strengths (1a; 1b; 6 Ch. 8)

There is no universal series of solvent strengths; relative elution
properties depend not only on the adsorbent, but in many cases on the
compound types being separated. In addition, there appears to be no uni-
form relationship between solvent property (dielectric constant, dipole
moment, e.g.) and eluting power; nor is there a relationship between
relative solubility of a compound and relative adsorbability. However, the
following empirical series have been found useful for most applications [in
order of increasing "polarity" (eluting power)--read down]. The entries
are those for which actual comparative measurements have been made.

For Alumina

Fluoroalkanes	Benzene	1-Pentanol
Pentane	Ethyl bromide	Dimethyl sulfoxide
Isooctane	Diethyl ether	Aniline
Petroleum ether (light)	Diethyl sulfide	Diethylamine
Hexane	Chloroform	Nitromethane
Cyclohexane	Methylene chloride	Acetonitrile
Cyclopentane	Tetrahydrofuran	Pyridine
Carbon tetrachloride	1,2-Dichloroethane	Butyl cellosolve
Carbon disulfide	Ethyl methyl ketone	2-Propanol
Xylene	1-Nitropropane	1-Propanol
Di-i-propyl ether	(Acetone)	Ethanol
Toluene	1,4-Dioxane	Methanol
1-Chloropropane	Ethyl acetate	Ethylene glycol
Chlorobenzene	Methyl acetate	Acetic acid

For Silica

Cyclohexane	Benzene	Ethanol
Heptane	2-Chloropropane	Water
Pentane	Chloroform	Acetone
Carbon tetrachloride	Nitrobenzene	Acetic acid
Carbon disulfide	Di-i-propyl ether	Methanol
Chlorobenzene	Diethyl ether	Pyruvic acid
Ethylbenzene	Ethyl acetate	
Toluene	2-Butanol	

For Magnesium Silicate (Florisil)

Pentane	Benzene	Methylene chloride
Carbon tetrachloride	Chloroform	Diethyl ether