Reminder: These notes are meant to supplement, not replace, the laboratory manual.

Thin Layer and Column Chromatography

History and Application:

Chromatography is a very old and widely used analytical and preparative technique. Chromatographic separations are used in forensics (CSI), research, chemical manufacturing plants, environmental testing (pesticides, air, water), water purification in remote locations and drug testing of athletes urine. It is one of the most common separation and identification methods used in the world.

Thin Layer Chromatography (TLC) is a quick, simple and inexpensive way to analyze small samples.

Column Chromatography is useful in isolating pure materials from mixtures. The sample size can vary from a few micrograms to tens of grams.

1. Here is some terminology related to this experiment:

Chromatography is a technique in which compounds in a mixture are separated based on differing affinities between a mobile phase and a stationary phase.

Mobile phase is a medium used in chromatography which moves through the stationary phase. In TLC and column chromatography, the mobile phase is an organic liquid. In Gas Chromatography the mobile phase is a gas.

Stationary phase is a material used in chromatography which does not move. The mobile phase passes through the stationary phase. The stationary phase is either a pure solid substance such as alumina or silica or a thin coating of liquid on a solid support or a gel.

In TLC the stationary phase is a spread in a thin layer on an inert support, usually a plastic sheet or a glass plate. The mobile phase moves upward by capillary action.

Partitioning is the separation of the compounds into distinct groups or assemblages. In chromatography this is commonly based on polarity and affinity between the mobile and stationary phases.

Affinity is a qualitative measurement of the attractive force between a compound and a phase. In chromatography this is commonly based on polarity. Materials with similar polarity will have a high affinity, or attractiveness for each other. Materials with a dissimilar polarity will have a low affinity for each other and will self separate.

Elution is the removal or exit of a compound from the stationary phase. In column chromatography this is when the material leaves the column.

Many compounds are not visible to the eye when dissolved in a solvent or adsorbed on a TLC plate. Visualization processes make these substances visible. Techniques used include UV lights that cause fluorescence or phosphorescence and chemical reactions that give colored compounds.

The R_f value in TLC is the ratio of the distance a compound moved from the origin to the distance between the origin and the solvent front.

TLC is used primarily as an analytical technique. It can be used to identify components of a mixture (by comparing Rf values), monitor the progress of a reaction (by comparing the intensity of spots) and check the purity of a sample (more than one spot indicates an impure sample). Rarely is TLC used to isolate and purify compounds into discrete samples.

Column chromatography is rarely used as an analytical technique, as TLC is. Column chromatography is primary used to separate relative large samples into pure components. Column chromatography is a preparative technique.

2. The mobile phase moves through the stationary phase and may carry with it some of the compounds. The ability of a solvent to carry and elute compounds from a column or TLC plate is related to the solvents polarity[i]. In general the stronger the eluting power the more polar the solvent. The stronger the eluting power, the quicker the compounds will exit the column or plate.

A ranking of the eluting power of classes[ii] of organic compounds can be made:
Acids>Alcohols > Esters> Ketones > Ethers> halogenated hydrocarbons> hydrocarbons

The eluting power of specific organic solvents are as follows.
Ethanol > ethyl acetate> acetone >MTBE> methylene chloride >toluene>heptane

Heptane is much less polar than MTBE. Hexane has a lower eluting power than MTBE.

Sometimes the desired polarity or eluting power of a solvent is difficult to achieve with a pure solvent and a mixture of solvents is used. The mobile phase for the TLC is a 30:1 Toluene ethanol mixture. The 1 part of ethanol in 30 parts of toluene slightly increases the eluting power of the solvent.

When separating two or more compounds via chromatography, it is very important to choose the correct solvent as a mobile phase. If too weak of an eluting power solvent is chosen, it will take a very long time and a very large volume of solvent to elute the compounds. If too strong of an eluting power solvent is chosen, all the compounds will elute immediately and no separation will occur.

3. The two most common stationary phases used in TLC and Column are alumina and silica gel. Both of these materials are exceedingly polar. Alumina is aluminum oxide or Al_2O_3.The surface of alumina is covered by oxygen atoms. Because of the small particle size[iii], a cubic centimeter of alumina may have a surface area of approximately 100 m^2. This means that there is just over half a volley ball court of surface area per milliliter of alumina. Silica is SiO_2. Silica has similar properties to Alumina.

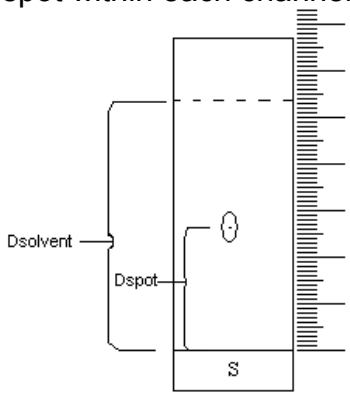
4. Separation occurs due to different affinities of the compounds within a mixture to the stationary and the mobile phase. Polar compounds easily dissolve in polar solvents and have a low affinity to nonpolar solvents. Compounds have a high affinity for solvents with a similar polarity to themselves. This can also

be paraphrased as like materials like other materials like themselves or "like like like".

Partitioning takes place as compounds within mixtures self-separate based on affinities.
In most column and TLC the stationary phase has a very high polarity and the mobile phase has a lower polarity. The compounds will partition based on their relative affinity to the mobile phase and the stationary phase. Alumina is a very very polar substance. Toluene:ethanol in a 30:1 ratio has a relatively low polarity. Ferrocene is less polar then acetylferrocene. This means that ferrocene will like, or have a higher affinity to the less polar mobile phase than the more polar stationary phase. In contrast acetylferrocene will have a larger affinity to the stationary phase (more polar) than the mobile phase. If a compound has a high affinity to the stationary phase it will come out slower than a compound which has a lower affinity to the stationary phase. Which compound, ferrocene or acetylferrocene, do you expect to elute the column first?

5. The Rf in TLC is the ratio of the distance the spot traveled to the distance the solvent traveled on a TLC plate. The higher polarity the compound, the larger affinity of the compound to the stationary phase and the smaller the R_f. The lower the polarity the compound, the higher the affinity to the solvent and the larger the R_f. If a solvent is changed from a low polarity solvent (like hexane) to a higher polarity (like ethylacetate) the eluting power will increase and all the Rf values will increase

To obtain a Rf measure the distance from the solvent start line to the middle of the spot. The distance between the smallest two lines on the ruler should be approximated. Measure the distance from the solvent start to the solvent front (where the solvent stopped moving). If more than one spot is present in a channel, calculate the R_f for <u>each</u> spot within each channel.

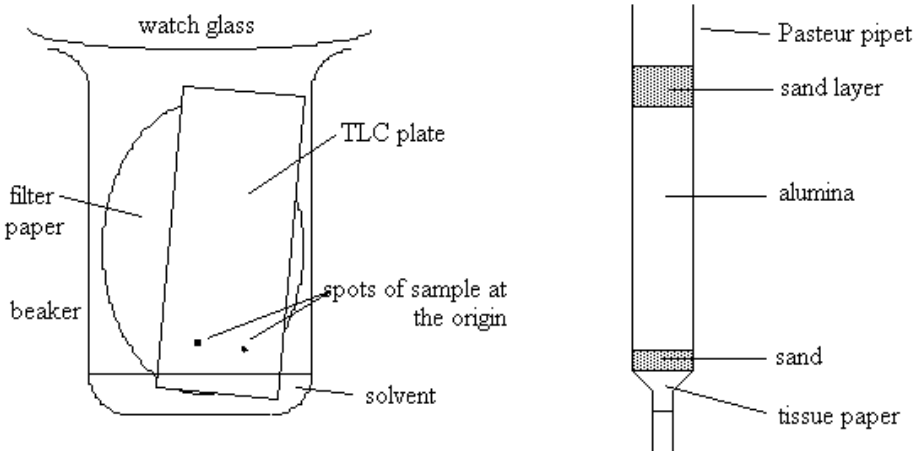


$$R_f = \frac{Distance\_spot\_traveled}{Distance\_solvent\_traveled} = \frac{D_{spot}}{D_{solvent}} = \frac{2.63cm}{5.38cm} = 0.489$$

The units cancel. R_f is a unit less value and will always be between 0 and 1, $0 \le R_f \le 1.0$. If R_f equals 0 the spot did not move at all, the compound is very polar and has little to no affinity to the mobile phase. If R_f value equals 1.00 the compound traveled right with the solvent, and the compound is very non-polar with little to no affinity to the stationary phase.

6. The TLC plates being used today are made up of a thin film of alumina on a plastic sheet. The alumina is approximately[iv] 250 μm (0.25 mm) thick. Handle TLC plates only by the edges. If a greasy finger is placed on the matte surface of the plate, the plate is ruined. The solvent or mobile phase being used in today's experiment is a 30:1 by volume mixture of Toluene and ethanol. The mobile phase will move up the TLC by capillary action. This same force is responsible for water creeping up a papertowel whose corner has been dipped in a puddle.

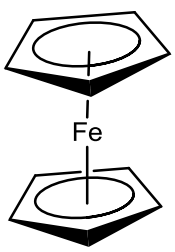
7. Here is the apparatus used for thin-layer and column chromatography. The TLC apparatus is on the left, and the column chromatography apparatus as used in this experiment is on the right.



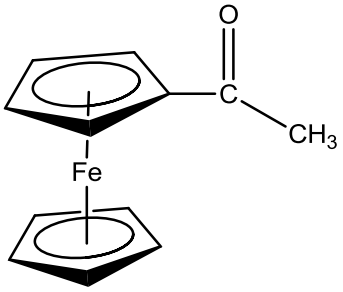
In the TLC apparatus immediately above, the solvent moves upward by capillary action, serving as the mobile phase. The plate has a layer of alumina gel on its surface, serving as the stationary phase. The filter paper is soaked with solvent and puts solvent vapors in the atmosphere inside the beaker, preventing evaporation of the solvent from the plate during the experiment. The watch glass or piece of aluminum foil keeps these vapors inside the beaker.

In the column chromatography apparatus, the top layer of sand prevents the solvent (mobile phase) from disturbing the adsorbent (which is the stationary phase) when it's poured in. The bottom layer of sand provides a level surface to keep the adsorbent layer uniform. The wad of tissue paper allows the mobile phase to flow through while preventing the sand from doing the same.

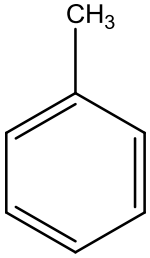
8. Here are the structures of the organic chemicals used in this experiment.



Ferrocene



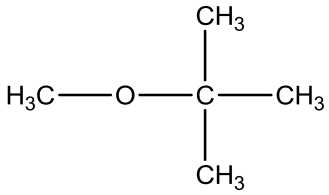
Acetylferrocene



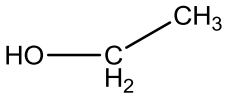
Toluene

CH_2Cl_2

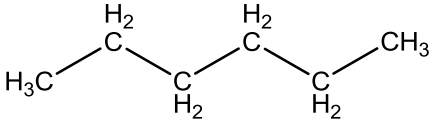
Methylene Chloride



Methyl t-Butyl Ether (MTBE)



Ethanol



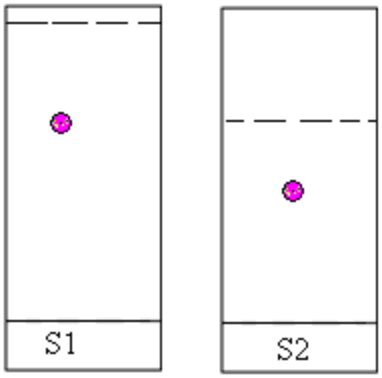
Hexane

Ferrocene and Acetylferrocene are organometallic compounds. This means that they contain both a metal atom and carbon atoms. These two compounds contain an iron atom bonded between two cyclopentadiene units.

9. Here are some pitfalls of TLC: If you use too much solvent in the bottom of the beaker, or if you carry the beaker around during the experiment allowing the solvent to slosh part way up the plate, the sample you are studying may wash off the plate into the solvent at the bottom. If you leave the watch glass off the top of the beaker, the solvent may evaporate from the plate, giving R_f values that are artificially high.

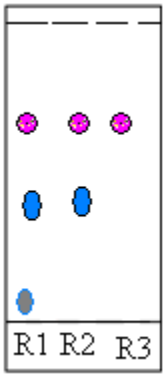
10. The most common error which occurs while running a column chromatography is if any part of the stationary phase is allowed to run dry. This will lead to the column support cracking and will result in poor separation or incomplete elution of the components.

11. TLC interpretation can be a little confusing at first. With practice it will become second nature. Each unique compound has a distinct Rf value in a given solvent system. This means that the Rf can be used to identify compounds.



Two reactions were run. Each sample was spotted on a TLC plate. Note that the absolute distance the spots moved are different. The solvent fronts moved different distances. Calculate the Rf for each. Do you believe these two samples contain the same compound?

Different compounds within a mixture usually have different Rf values and hence will be seen as different spots. The number of spots in a given channel can be used to determine the number of different compounds in that mixture.



A reaction was run three different times. The reaction mixtures were spotted on a single TLC plate and developed. The results are above. How many different compounds are located in reaction mixture number 1? 2? 3?

12. Safety Precautions[v],[vi]

Ferrocene causes irritation to the eyes and skin. Prolonged ingestion or inhalation may cause liver damage. Acetylferrocene is toxic if ingested or absorbed through skin. Do not eat or breathe these materials. Keep away from skin. Wear gloves. Wash hands at end of lab.

Dichloromethane (Methylene chloride) is a chlorinated organic material. Most chlorinated organics possess some negative health effects. High level exposure to dichloromethane can cause central nervous system depression; long term exposure may cause tumors. Handle in small quantities, minimize contact with liquid and avoid breathing vapors.

Toluene, ethanol, Hexane and methyl t-butyl ether (MTBE) are flammable organic liquids. Have no flames in lab. Avoid breathing vapors.

[i] Experimental Organic chemistry, 2nd Ed, J. C Gilbert & S. F. Martin, Sanders New York 1994, P157
[ii] Quantitative Chemical Analysis, D. C. Harris W.H Freeman 1982 p584
[iii] Sigma –Aldrich Chemical Company Product specifications
http://www.sigmaaldrich.com/etc/medialib/docs/Sigma-Aldrich/Product_Information_Sheet/a1772pis.Par.0001.File.tmp/a1772pis.pdf (September 14, 2011)
[iv] Fisher Scientific Catalogue Product Specifications http\\www.fisher.sci.com (September 14, 2011)
[v] Fisher Scientific MSDS sheet Ferrocene http://fscimage.fishersci.com/msds/03388.htm and Acetylferrocene https://fscimage.fishersci.com/msds/69220.htm (September 14, 2011)
[vi] Fisher Scientific MSDS for Dichloromethane methylene chloride MSDS (September 14, 2011)