

You will separate earth from fire,
The subtle from the compact,
Gently with great skill.
Thus you will possess the glory of the whole world
And all obscurity will flee from you.”

Alchemist verse from “Thin Layer Chromatography” by Hamilton & Hamilton

Overview of the first four technique labs:

The first four technique experiments represent a progression necessary to separate compounds from a mixture, purify the individual compounds and identify each component. Each step helps you gather clues as to the identity of your unknowns but you cannot (should not) make a final judgment until you have determined the melting point of the pure unknown compound in step 4.

Step 1 – Thin Layer Chromatography

We will begin to look at our mixture with Thin Layer Chromatography in order to ascertain how many different compounds are present in the mixture. We will also learn about the compound's polarity characteristics. In addition we will make some tentative identification of the unknown compounds by comparing them to a series of known compounds. Each mixture will have at least one compound that matches one of the known compounds.

In other words you will not be able to unequivocally identify your unknown compounds as a result of step 1!

Step 2 – Liquid-Liquid Separation

You will separate your unknown compounds on the basis of their solubility characteristics. The acid/base behavior of your unknowns should give you additional clues as to their identities.

Step 3 - Recrystallization

You will purify your unknown compounds based on their solubility characteristics. The physical aspect of your purified unknowns (the color and texture of the crystals) may give you additional clues as to their identities.

Step 4 – Melting points

You will (finally) identify your unknown compounds by their melting points and by comparing their melting points with known compounds.

“He shall separate them one from another, as a shepherd divided his sheep from the goats.”
The Holy Bible, St. Matthew 25:32

Background:

Real-life organic chemistry is the laboratory often begins with separation and purification of organic compounds from a mixture. For example, plants contain hundreds and maybe thousands of organic compounds. If you want to isolate a particular organic compound, such as the Vitamin A precursor carotene, from a plant such as carrots you need to separate carotene from all the other compounds that are present in the carrot.

Adsorption Chromatography is the most useful and versatile means of separating compounds to purify and identify them. One of the earliest forms of chromatography to be developed is called thin layer chromatography (TLC). Indeed, the principles of TLC can be demonstrated by spilling a bit of water on an ink-written page.

The first component of TLC is the adsorbent. The adsorbent is a porous material that can adsorb (suck up) liquids. Paper, silica gel and alumina (aluminum oxide) are well-known TLC adsorbents. However, charcoal dust, powdered sugar and even cellulose can be used as adsorbents. Silica gel and alumina are attached in a thin layer to a solid support such as a glass or plastic slide. Paper is a self-supporting adsorbent.

When you dip one end of the TLC slide in a solvent the solvent will travel up the plate by adsorbing to more and more adsorbent as it goes. This is an example of capillary action – the solvent is more attracted to the adsorbent than it is to itself, thus it will climb up the adsorbent against the pull of gravity.

The mixture of compounds you want to separate on your TLC plate is called the solute. The solute is attracted to both the solvent and the adsorbent. As the solvent travels up the TLC plate it carries the solute along with it. However, different compounds in the solute stick to the adsorbent so they tend to lag behind the solvent front as it travels up the plate. How well they stick to the adsorbent depends on their polarity.

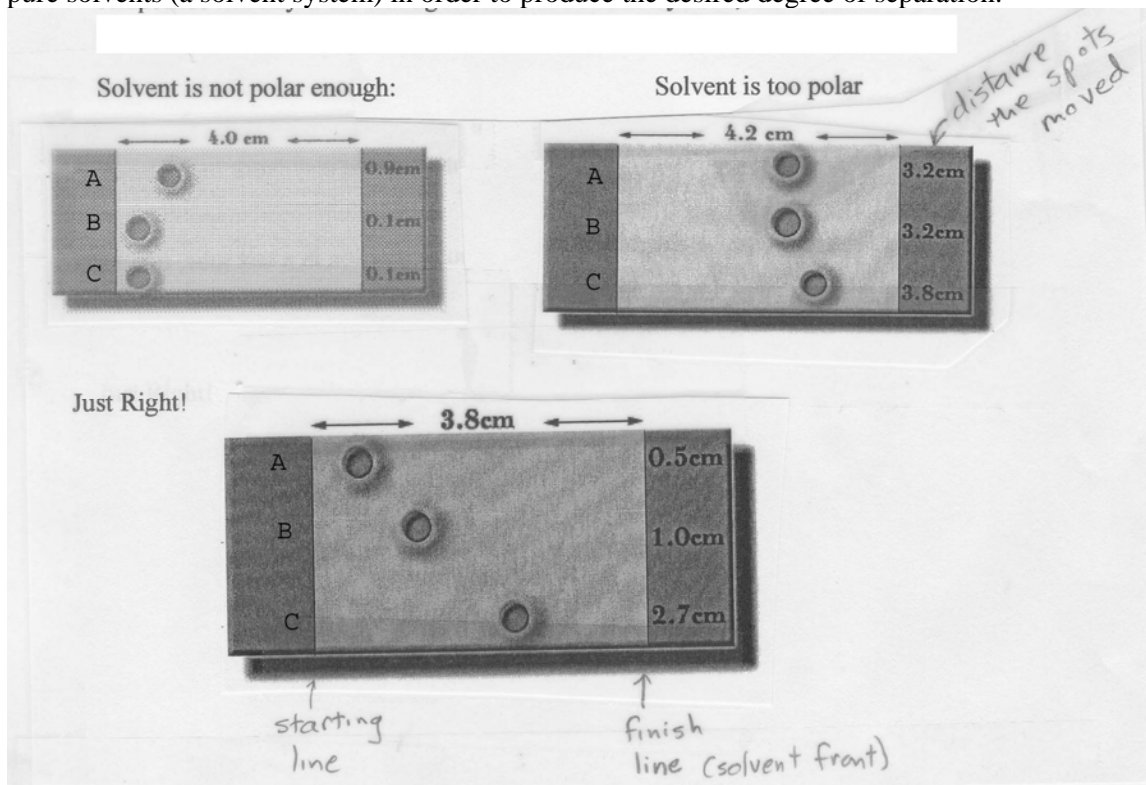
In general, the more polar a compound is the better it will stick to the adsorbent and the slower it will travel up the plate. In the language of TLC, polar compounds have low R_f values (close to zero). Nonpolar compounds do not stick to the silica gel very well and tend to run along close to the solvent front. In the language of TLC, nonpolar compounds have high R_f values (close to one). Thin Layer Chromatography, therefore, separates compounds by differences in their polarity.

“Good-night, good-night? Parting is such sweet sorrow That I shall say good-night till it be tomorrow.”
William Shakespeare, Romeo and Juliet

On the other hand, solvent polarity also plays a role. In general, the more polar a solvent is the faster it will pull along compounds less polar than itself. Rating common solvents on the basis of their polarity would look like this:

| Solvent | Relative Polarity |
|-----------------|-------------------|
| Petroleum ether | Least polar |
| Hexanes | |
| Dichloromethane | |
| Ethyl Acetate | |
| Acetone | |
| Ethanol | |
| Methanol | |
| Water | Most polar |

The TLC Challenge is to find the right combination of solvent and adsorbent that will spread out the compounds in the mixture you are separating. If the solvent is too polar, all the compounds will travel along with the solvent front and not separate. If your solvent is not polar enough the compounds will stay at the starting line and not move. Very often, one has to use a mixture of pure solvents (a solvent system) in order to produce the desired degree of separation.



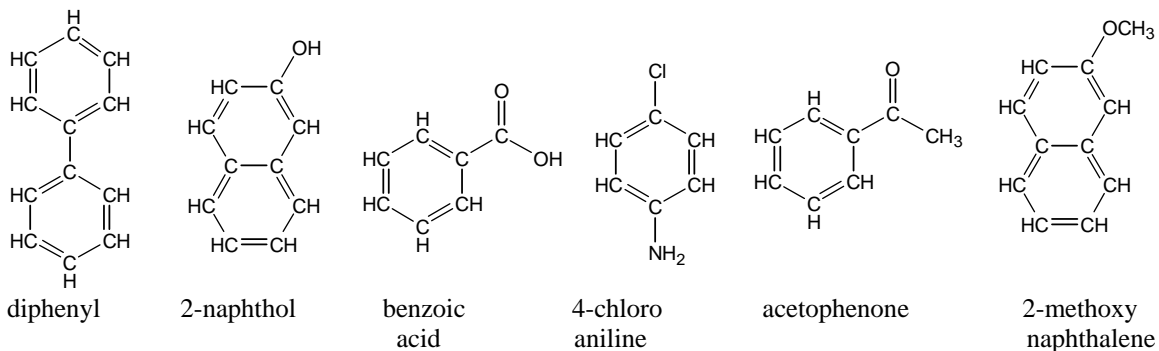
Compliments of Eric Stachurski, Organic Chemistry, Fall 2003

James Zubrick in "The Organic Chem Lab Survival Manual" gives a very nice introduction to chromatography in chapter 26 and a detailed description of TLC techniques in chapter 27.

See also "Thin-Layer Chromatography: The 'Eyes' of the Chemist: Dickson, Kitteredge, & Sarquis. In Journal of Chemical Education Vol. 81 (2004) No. 7 p. 1023

Introduction:

In this experiment you will explore the relative strengths with which different compounds are adsorbed by silica gel Thin Layer Chromatography (TLC). You will employ a series of substituted benzenes:



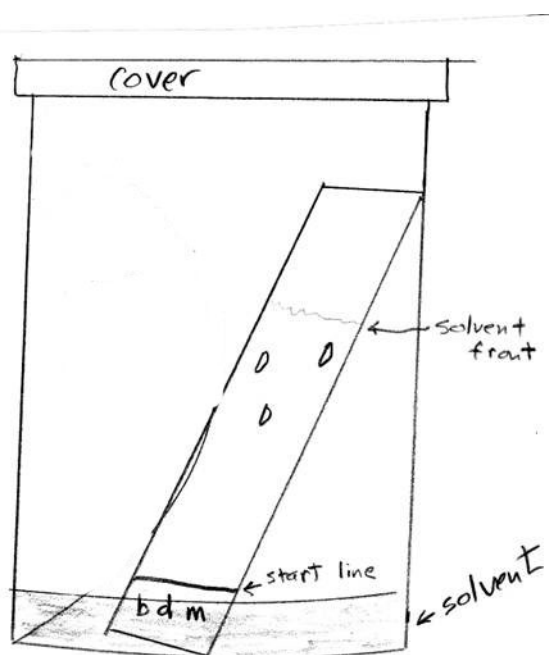
Since the compounds are colorless, it will be necessary to view the spots utilizing UV light and iodine vapor adsorption (in that order).

Procedure:

Obtain at least 8 plate spotting capillary tubes. (One for each compound and one for the mixture of unknowns.)

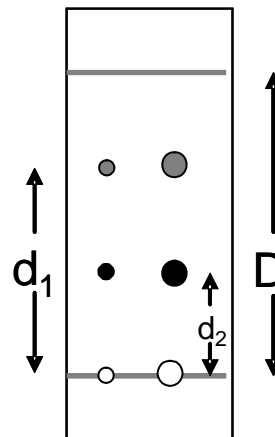
Set up three developing chambers (empty baby-food jar with a 7 cm filter paper circle on the inside).

Obtain 6 plastic-mounted 2.5 x 7.5 cm silica gel slides with fluorescent indicator.



Part I.

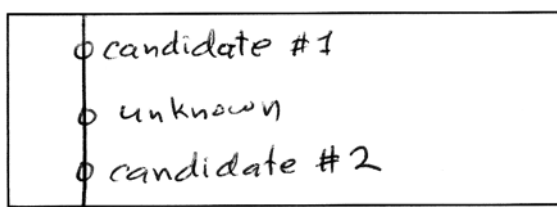
1. Carefully label 6 small test tubes, put a few drops of each benzene derivative into the appropriate test tube. **You may share your test tubes with a lab partner.**
2. Set up a developing chamber: a glass jar.
3. Pour about 0.5 cm of the developing solvent (hexane, dichloromethane or methanol) in the jar. The solvent must cover the bottom of the jar. Replace the cap on the jar and allow the solvent to permeate the filter paper.
4. Obtain two silica gel slides. Place the silica gel slide on a clean surface. On the silica gel side (the powdery side) of the slide draw a faint line in pencil about one centimeter from the bottom of each of your slides.
5. Use separate capillary tubes for each benzene derivative. First load your capillary with the benzene derivative by dipping it into the benzene derivative solution. Touch the solution-containing capillary lightly to the silica gel slide by touching it to a spot on the pencil line about 0.5 cm from the left side of the slide. That way, you can spot three benzene derivatives on one slide.
6. Spot two pre-prepared silica gel slides with the compounds (three spots to a slide).
7. Carefully place the slide in the developing chamber. Handle your slides with a tweezers as much as possible – avoid touching the silica gel with your fingers. Only one slide in a developing chamber at a time.
8. The slide takes a few minutes to develop. You will be able to see the solvent climbing the slide.
9. When the solvent front is about 0.5 cm from the top of the slide it has reached the finish line. Take the slide out of the chamber. Mark the solvent front with a light pencil line.
10. Lay the slide on a paper towel and let the solvent evaporate. When the solvent evaporates, view slides under UV light. Lightly circle the spots with pencil.
11. Place the slides in the iodine vapor chamber. Take the slide out when the spots become visible. (You only have to develop one or two slides with iodine.)
12. Calculate the R_f values for each compound in your solvent. The R_f value is the distance between the starting line and the center of the spot (d_1 or d_2) divided by the distance between the starting line and the finish line (D). **Share your results with your lab partner.**
13. You should have data for all six compounds in at least three different solvents. Enter your data on the class data excel spreadsheet before leaving the lab.
14. Thinking outside the box: Perform a TLC experiment in a mixture of solvents (such as 50% hexane 50% dichloromethane). Be sure to record the proportions of your solvent mixture.



Part II.

You will utilize your knowledge of TLC to: (1) find a solvent that will separate the components of an unknown mixture and (2) tentatively identify the components of a mixture by the technique of co-chromatography.

1. Obtain a vial of unknown mixture from the instructor. Dissolve a small quantity (the size of a BB) of it in about 0.5 mL (10 drops) of dichloromethane in a small test tube.
2. Use your developing chambers from Part I.
3. Spot a slide from left to right with (1) one of the known compounds from Part I. (2) your unknown mixture (3) another known compound.



4. Your primary objective in steps 1-3 is to find a solvent that will separate the components of your mixture. You will begin to gather clues as to the identity of one or both of your unknowns through this process.
5. Develop the slide in your selected solvents. Visualize the spots under UV. Let the results from this set of slides guide your choice of solvent for the subsequent trials. For example, if the R_f value is high and poor separation occurs, decrease the polarity of the solvent slightly. If the R_f value is low and poor separation occurs increase the polarity of the solvent slightly. You may mix solvents in known ratios in order to "tweak" the results.
6. Spot and develop two or three more slides trying to achieve maximum separation of the components as well as a match for the unknown compounds.
7. You should have developed at least 3 slides with your unknown compound between two known compounds.

Not all of the known compounds correspond to the unknowns. You not expected to have identified all of your unknown compounds at the end of this experiment. You will have 3 more experiments with which to determine the exact identity of your unknowns.

Dispose of your left-over solutions and solids properly.

There will be a solvent disposal container in the fume hood for liquid wastes.

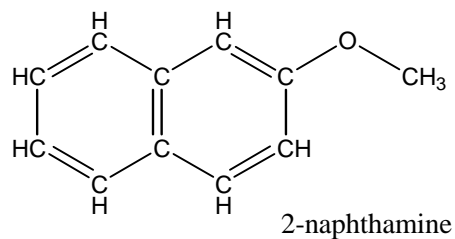
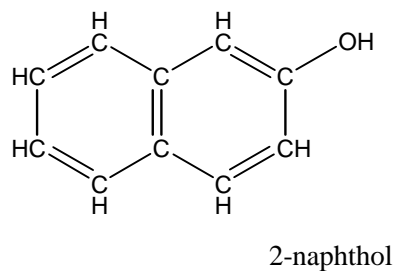
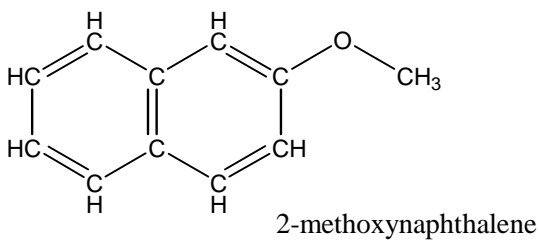
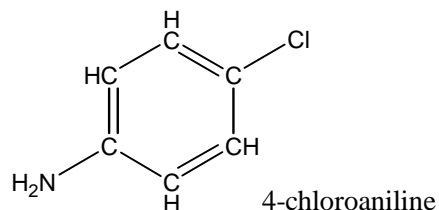
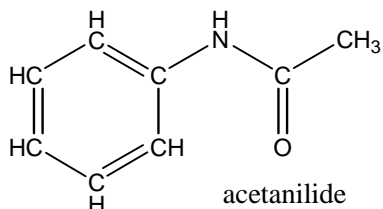
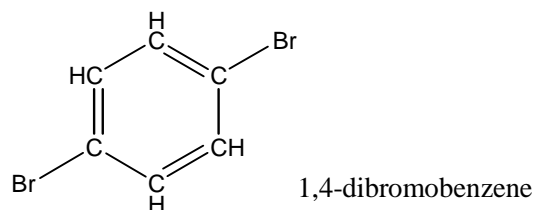
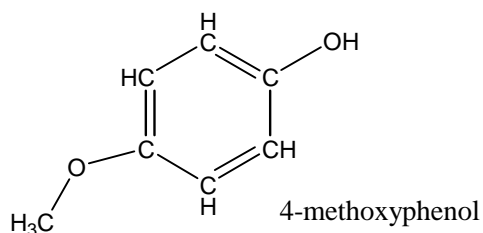
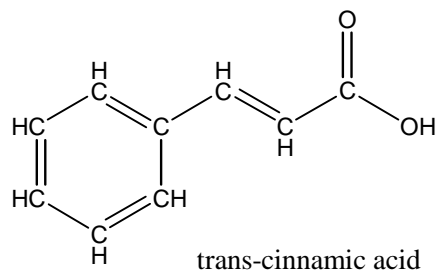
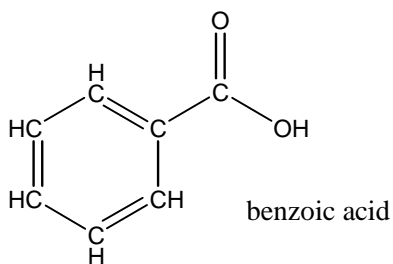
Filter paper and extra TLC plates (don't waste them!) can go in the solid waste (fume hood).

Used capillary tubes can go in the solid waste (fume hood).

Data entry:

In organic lab this year we will make class data available to the whole class. Before you leave the lab you will record your TLC data (R_f values for compounds in a particular solvent or solvent mixture) on a laptop computer. This information will be made available to you on the chemlab website. You will want to consult this data when you write your lab report.

unknowns for experiments 1,2,3 & 5



Check list for completing the "Prelab" section:

_____ Read through the appropriate section of the syllabus

_____ *Title.*

_____ *Purpose.* Refer to "Introduction"

Physical constants. Create a table of physical constants and safety data.

| Name | Formula | M.W. g/mole | m.p. °C | b.p. °C | Density g/mL |
|-----------------------|-----------------------------------|----------------|---------|---------|---------------------------------|
| Aceto phenone | C ₈ H ₈ O | 120.15 | 19.6 | 201.7 | 1.026 |
| 4-Chloro aniline | | | | | |
| Benzoic acid | | | | | |
| Diphenyl | C ₁₂ H ₁₀ | 154.21 | 69-71 | 255 | (don't need density for solids) |
| 2-methoxy naphthalene | C ₁₁ H ₁₀ O | 158.20 | 73-75 | 274 | (don't need density for solids) |
| 2-naphthol | | | | | |
| Dichloro methane | | | | | |
| Hexane | | | | | |
| Methanol | CH ₄ O | 32.04 | -98 | 64.6 | 0.79 |
| Iodine | I ₂ | 253.81 | 114 | 184.4 | (don't need density for solids) |

| Name | Solubility | Safety Information |
|-----------------------|---|--|
| Aceto phenone | Some soluble in water, soluble in alcohol and nonpolar solvents | Somewhat toxic. Irritating to eyes. |
| 4-Chloro aniline | | |
| Benzoic acid | | |
| Diphenyl | Insoluble in water, soluble in alcohol and nonpolar solvents | Moderately toxic. Irritating to eyes, skin and respiratory system. |
| 2-methoxy naphthalene | Insoluble in water, some soluble in alcohol, sol in nonpolar solvents | Moderately toxic. Irritating to eyes, skin and respiratory system. |
| 2-naphthol | | |
| Dichloro methane | | |
| Hexane | | |
| Methanol | Miscible with water, alcohol and nonpolar solvents | Flammable Slightly irritating to skin and eyes. |
| Iodine | Some soluble in water, soluble in alcohol and nonpolar solvents | Harmful vapors. Avoid contact with eyes. |

References: 1)
2)
3)

Structures and equations.

_____ Draw the Lewis structures of dichloromethane, hexane and methanol

_____ *Flowchart.* Refer to "Procedure"

Calculations.

_____ Do your best to arrange the benzene derivatives in part I from the least polar (1) to the most polar (6). Generally, the compounds with the most polar bonds are the most polar compounds.

| Benzene Derivative | Relative Polarity |
|---------------------------|--------------------------|
| 1) | Least polar |
| 2) | |
| 3) | |
| 4) | |
| 5) | |
| 6) | Most polar |

Safety Question:

Your hood neighbor has a mild spaz attack and knocks over his/her developing chamber with dichloromethane in it. The dichloromethane spills in the hood. How should you react to this lab accident? How could lab accidents like this be prevented?

Experimental Observations and Data

You will hand a copy of your Experimental Observations and Data before you leave lab.

_____ Read through appropriate section in the syllabus.

Experimental Observations.

- ___ Is it written in passive voice past tense. "The TLC slide was developed in hexane."
- ___ Did you clearly state which compounds were developed in which solvent?
- ___ About how long (nearest minute) did it take to develop a slide in hexane?
- ___ About how long (nearest minute) did it take to develop a slide in dichloromethane?
- ___ About how long (nearest minute) did it take to develop a slide in methanol?
- ___ What were the colors of the different solutions and solids including the unknown?
- ___ What do TLC plates look like under UV light?
- ___ What did you observe in the iodine chamber?
- ___ Sketch out the result of each TLC plate.
- ___ Any blunders to report? Did you put your slide in the developing chamber upside down?
- ___ Did you record interesting sights and smells? Did anything weird or unexpected happen?

Data:

- ___ Include all raw data please.
- ___ Calculate the R_f values from one slide. You don't have to do all the R_f values right now.
- ___ Clearly show which data you collected yourself and which you borrowed.

Lab Report Checklist:

___ Read through appropriate section in the syllabus.

Results.

___ Table of: compound - solvent system - R_f value - comments (shape of spot) For example:

| Compound (include unknown) | R_f value in Hexane | R_f value in CH_2Cl_2 | R_f value in Methanol | R_f value in 50% hexane 50% methanol | Comments |
|-------------------------------|-----------------------|---|-------------------------|--|----------|
| | | | | | |
| | | | | | |
| | | | | | |

Discussion and Conclusion.

___ How did your results compare with the class results this year?

___ How did your results compare with the class results from last year?

___ Compare your predicted order of elution (on your prelab) to your experimental results.

| Compound | Experimental polarity | Predicted polarity |
|----------|-----------------------|--------------------|
| | 1) Least Polar | |
| | | |
| | | |
| | | |
| | 6) Most Polar | |

___ Explain how you determined the order of polarity in the previous question.

___ What can you conclude about the polarity of different functional groups? Give specific examples. Were there any that surprised you?

___ Which of the following statements represent advantages of TLC? Choose all that apply.

- Spots tend to enlarge and change shapes as they move up the plate.
- The fluorescent indicator allows one to visualize almost any organic compound.
- Two different compounds may have very similar R_f values.
- One can see individual components of a complex mixture.

___ Green chemistry question: Organic chemistry labs tend to use large quantities of solvents such as dichloromethane, hexane and methanol. What are two practices that can be adopted to minimize the (harmful) impacts of solvent use on the environment?