THIN LAYER CHROMATOGRAPHY

OBJECTIVE

In this laboratory you will separate spinach pigments using thin layer chromatography (TLC).

INTRODUCTION

Mixtures of compounds are very common in Organic Chemistry. Most reactions produce more than one product. Naturally occurring materials are only rarely 100% pure. It is therefore desirable to have a simple, fast and efficient way to determine the purity of Organic mixtures. The separation of a mixture by passing it, in solution, over an adsorbent (such as Alumina or Silica Gel) is the basic idea of Chromatography.[1]

Chromatography is a very general phenomenon. It involves the passage of a mobile phase across a stationary phase[2] in a column. Usually a mixture of compounds is present in the mobile phase.[3] As soon as the mixture comes in contact with the stationary phase, some or all of the components of the mixture are adsorbed on it. As additional mobile phase comes along, some or all of the mixture will dissolve and continue moving. This adsorption/solution process continues along the length of the column. If a proper choice of mobile phase, stationary phase, solvent and other operating parameters was made, the mixture will be separated in the column and its various components will emerge at different times.

In Thin Layer Chromatography ("TLC"), a liquid solution is directly applied to a solid adsorbent. Capillary action draws a developing solvent up the TLC plate. As this solvent passes through the spot, the mixture will be dissolved and will begin to move with the solvent front. However, the adsorbent will also reabsorb part or all of the mixture. As more solvent comes by, the mixture will again go into solution, move further and be reabsorbed. Since different materials will be dissolved and reabsorbed at different rates, separation will take place. The slide is removed from the chamber once the solvent front reaches a predetermined spot near the edge farthest from the point of spotting. This passage of the solvent front through the adsorbent is known as **developing** the plate. The extent of separation, measured by retention factor ("Rf") value differences, will depend on the relative solubilities and relative strengths of adsorption of the components of the mixture.

Organic compounds interact with absorbents by a variety of interactions. If the compound is non-polar, it can only have weak 'Van der Waals' attractions for the absorbent. However, more polar molecules may interact more strongly by a variety of mechanisms including dipole-dipole interactions, coordination, and hydrogen bonding. The most important rule of chromatography is that *the more polar compounds will be absorbed most strongly on absorbents (stationary phases), while non-polar compounds will be only very weakly absorbed.*

In a typical chromatography experiment, the non-polar compounds, since they are poorly ab-

[1] http://en.wikipedia.org/wiki/Chromatography
[2] http://en.wikipedia.org/wiki/Stationary_phase_(chemistry)
[3] http://en.wikipedia.org/wiki/Chromatography

sorbed, will be held least strongly and will move quickly through the plate. Polar compounds, on the other hand, will be slowed on their process through the plate by their strong interactions with the solid phase. This separation based on polarity will explain most of the chromatography encountered in this course.

Types of Adsorbents used in Chromatography

Listed in decreasing power of adsorption:

Alumina
Activated Charcoal
Magnesium Silicate
Silica
Starch

Just as we have a variety of stationary phases to choose, we also have an even larger assortment of mobile phases (or, eluting solvents). They are also categorized according to their ability to move polar compounds through the chromatography column

Solvents Commonly Used in Chromatography

Listed in decreasing polarity:

Acetic Acid
Water
Methanol
Acetone
Ehtyl acetate
Diethyl ether
Chloroform
Methylene chloride
Toluene
Cyclohexane
Petroleum ether

For a typical separation, a variety of different combinations of solvent and adsorbent may be effective. However, these combinations are only obtained by trial and error, based on experience. There is *no* magic formula that will allow prediction of just the right set of conditions for any given separation.

Once you have developed your plate, since most compounds are colorless, the location of the separated samples, or spots, is usually not apparent. The plate must be **visualized**. This visualization may be accomplished in a number of ways. If the compound(s) fluoresce, shining a UV light on the plate may indicate the location of the separated spots. Conversely, the adsorbent may be made to contain a small amount of a fluorescing substance. When the developed plate is exposed to a UV lamp, most of the plate will fluoresce one color. Wherever a spot is located there will be either a different color or less fluorescence. While the UV light is ON, the position of the visualized spots is sketched on the plate with a sharp pencil.

Alternatively, visualization may be accomplished by reacting the developed plate with a chemical reagent. Iodine (I_2) is one of the easiest to use of the several common chemical visualizing agents. The developed slide is simply exposed to I_2 vapors in a chamber similar to the developing chamber for a few minutes. Almost all compounds will form a weak colored complex with the I_2. This complex will appear as a darker area on the slide. Again, the darkened areas are traced with a pencil before the I_2 evaporates and the color disappears.

This TLC technique usually requires only a few minutes for a complete analysis, and requires only about 10 microliters of the solution to be analyzed (a microliter is a millionth of a Liter (10^{-6} L, or 10^{-3} mL)). A few mL of the developing solvent is placed in a simple chamber, such as a 4-oz wide mouth jar. To insure an atmosphere saturated with the developing solvent in the chamber, a piece of filter paper is also present to act as a wick and the chamber is kept capped except when adding or removing a TLC plate.

The 'spots' are characterized by their R_f value[4] , a measure of how far the spot traveled with that combination of adsorbent and solvent. Rf values will change when either of these factors is changed.

$$R_f = \frac{\text{Distance spot traveled}}{\text{Distance Solvent Traveled}} \tag{1}$$

On this scale, TLC is only an analytical tool, albeit a very valuable one. If samples of the separated materials were desired, the entire experiment could be scaled up to allow milligrams to be separated. The plates would be larger and the amount of adsorbent would be increased, but the procedure would be the same. The spots would be visualized by UV (non-destructive) and then separately scraped from the glass plate. The samples could be recovered from the adsorbent by extracting the scrapings with a pure solvent such as ether, and then carefully evaporating the solvent.

PRE-LAB

1. Briefly outline the procedure (bring your outline with you to the lab)
2. Answer all assigned questions.

Questions

Question 1: Which of the following pairs of compounds would be most easily separated by thin layer chromatography: n-octyl alcohol and 1-octene, hexadecane and octadecane, or chlorobenzene and bromobenzene? Justify your answer.

Question 2: What pigments can you expect to find in the spinach? (You can easily find the answer to this question by searching on the Internet)

Question 3: How could thin layer chromatography be used to aid in the identification of a compound?

[4] http://en.wikipedia.org/wiki/Retardation_factor

Question 4: The following items are not allowed in the labs:

Backpacks
Calculators
Food and drink

Question 5: The following items are required lab attire:

Long pants, covered shoes
Long pants, sleeveless shirts
Shorts, tank tops

Question 6: Table of physical constants

PROCEDURE

CAUTION: Pentane is highly flammable. Perform only in a well ventilated hood.

Obtain a TLC plate and a developing chamber. In the developing chamber, place a piece of filter paper to serve as a wick and about 10mL of the TLC developing solvent (7:3 mixture of hexane:acetone). Be sure the depth of solvent is *no more than half* of the distance from the end of a TLC plate to the 'start line' (no more than 0.5 cm). If the start line should ever touch the solvent directly, the TLC experiment is ruined since some or all of the sample will be dissolved into the solvent pool. Stopper the chamber to allow it to become saturated with solvent vapors. A cap is kept in place *at all times*, except when adding or removing a plate. Allow at least 5-10 minutes (with occasional gentle shaking) for the chamber to equilibrate before the first plate is developed.

Figure 1

Trace a small pencil line about 1 cm from the bottom of the plate and another small pencil line

5cm up the plate from that line. You should make your pencil marks on the papery side (not on the side with the glossy finish). Add 3 mL of spinach juice and 6mL of pentane to a large stoppered test tube and shake vigorously for 1 minute. Spin the mixture in a centrifuge for 5 minutes, after which time a transparent green top layer should be visible.

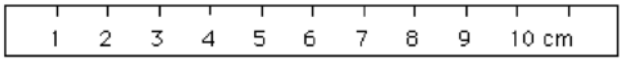
Transfer the transparent green top layer using a Pasteur pipet to a clean 50 mL beaker. Evaporate the pentane by heating the beaker on a hot plate at a low heat setting (about 95*C) for a few minutes until only a few drops of liquid remain. If almost all of the solvent is accidentally evaporated, two or three drops of pentane may be added to redissolve the green residue. Adding a drop or two of pentane after evaporation will ensure better loading of the TLC plate.

Use a microcapillary tube to load the extract onto the TLC plate. Allow the tip of the drop at the end of the capillary to just touch the plate. Blow lightly onto the plate after each drop is added to allow the solvent to evaporate. Each time make sure the spot never gets any larger than it did the first time. This will insure a very concentrated spot at the start line and will give the most concentrated spots (nearly round) on development of the plate.

After all spots have been applied, and all spots are dry, the plate may be placed into the developing chamber and capped immediately to avoid loss of the solvent saturated atmosphere. Almost immediately, the solvent will begin to migrate up the plate. Once the solvent reaches the top line on the plate, remove it and allow the plate to dry. As the plate dries, you will notice a change in its appearance. However, the spots will not be visible unless they are colored materials. The spots must be *visualized*. A UV lamp is the simplest way to visualize. Place the dry plate on the bench top and allow the UV light to shine on it. If there is a spot, it will probably show as a different color of fluorescence than the background, or as a darkened area on the adsorbent.

CAUTION: Never allow UV light to shine on anyone's eyes! Permanent eye damage may result. Be extra careful to keep the UV lamp pointed down at the bench top at all times.

With a very sharp pencil or other sharp instrument, draw an outline of each spot in the adsorbent. Turn OFF the UV lamp and carefully put it away. Include your TLC plate with your lab worksheet, and label the pigments to the side of your plate.



TLC Ruler (cm.)

Figure 2

IN-LAB QUESTIONS

Download and print the following worksheet. You will use this worksheet to record your answers to the In-Lab questions.

Questions

Question 1: How many pigments were you able to visualize and identify? List them and describe their appearance.

Question 2: What is the Rf value for each of the pigments in your TLC plate?

Question 3: Where your RF values comparable to those obtained by other students in the class? If not, could you explain any observed discrepancies?