

## EXPERIMENT 3

### THIN LAYER CHROMATOGRAPHY

#### A. PRELAB ASSIGNMENT

Prepare a Table of Physical Constants for the following compounds.

Ferrocene	Aspirin
2-Nitroaniline	Salicylamide
4-Nitroaniline	Caffeine
2-Nitrophenol	Acetaminophen

(Consult the Merck Index as well as the Handbook of Chemistry and Physics).

#### B. THEORETICAL BACKGROUND

The theory of chromatography will be discussed in the laboratory lecture. However, a few points do require emphasis. The most often used stationary phases, silica gel and alumina, are polar materials. Consequently, the least polar compound will have the highest  $R_f$  value. Increasing the polarity of the eluting solvent will increase the  $R_f$  values of all compounds, but **will not** change the order of the  $R_f$  values. If the solvent is too polar, all compounds will follow the solvent front,  $R_f = 1.0$ . Too low a solvent polarity will leave all compounds at the origin,  $R_f = 0$ . Neither of these situations is useful in analyzing mixtures. Usually, the polarity of the eluting solvent can be adjusted by mixing solvents of different polarities to yield  $R_f$  values between 0.1 and 0.9. Under all conditions, spots of identical compounds should yield identical  $R_f$  values. However, in some situations, different compounds can also yield identical  $R_f$  values. Therefore, confirmation of the identity of a compound by TLC requires that the compounds be analyzed against standard compounds under several sets of conditions.

#### A. GENERAL PROCEDURES

##### 1. Application of Sample

It is best for samples to be applied as small (2-3 mm diameter) spots.

During development of the plate, the spots tend to diffuse, so that the final

spot is considerably larger than the original spot. The larger the final spot, the more difficult it is to resolve a mixture into completely separate spots. Small spots can be obtained by using fine capillary tubes as applicators. Compounds or mixtures are applied approximately 1 cm apart in an even line about 1 cm from the bottom of the TLC plate.

## **2. Development of the Plates**

The eluting chamber must be saturated with the eluting solvent vapor. To assure vapor saturation, a piece of solvent-saturated filter paper is used as a liner for the chamber. The level of solvent in the bottom of the chamber must be below the positions of the spots on the plate. Too high a solvent level will wash the spots off the plate, preventing any meaningful analysis. The plate is placed into the chamber and the solvent is allowed to rise up to within 0.5 cm of the top of the silica gel coating. When this occurs, the plate is removed from the developing chamber and the extent of solvent migration (solvent front) is marked on the plate right away.

## **3. Visualization of Spots**

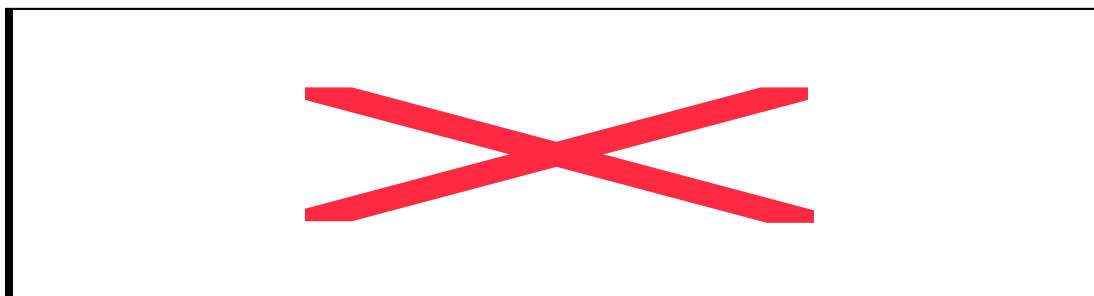
Colored compounds are easily located on TLC plates. However, the vast majority of organic molecules are white or colorless, and thus they are invisible on TLC plates. In such analyses, some method must be used to make the spots visible. Although several techniques are available, one of the simplest and most used methods is the  $I_2$  (Iodine) visualization technique. In this process, the TLC plate is placed in an iodine chamber. Most organic compounds adsorb yellow-brown  $I_2$  molecules. Thus, spots of chromatographed compounds appear as yellow brown spots after the plate has been treated with  $I_2$ . When the plate is removed from the  $I_2$  chamber, the  $I_2$  will gradually diffuse away from the spots, so the plate should be immediately marked to indicate the positions of spots. Another method used to visualize the spots is to observe the plate under UV light. Most conjugated compounds appear as violet or purple spots under UV light, if the coating on the plates contains a UV absorber.

#### 4. $R_f$ Values

$R_f$  values for many compounds are published for specific sets of conditions (sample quantity, solvent system, temperature and coating material). Often these conditions are difficult to duplicate, so the best way to identify a compound in a mixture is to compare the behavior of the mixture and a standard on the same plate. Even then, variations in the thickness of the coating can cause significant variation of  $R_f$  values on the same plate.

#### 1. Analysis of a Mixture of Colored Compounds

Colored Standard Compounds



2-nitrophenol (2-np)

2-nitroaniline (2-na)

4-nitroaniline (4-na)

ferrocene (fe)

##### a. Choice of Sample Volume

Transfer small samples (several drops) of the four standard colored solutions to small plastic vials.

Choose the lightest colored standard solution for this part of the experiment. Spot this standard on the TLC plate in increasing order of applications (e.g. 1 for position 1, 2 for position 2, etc.). Develop the plate using a 3:2 mixture of hexane and acetone. From the appearance of the final spots, choose the appropriate number of applications for your subsequent plates with the colored compounds.

b. Identification of Unknown Compounds

For your initial analysis, spot your unknown and the colored standard compounds on one plate. Be sure to note which spot corresponds to which compound.

Develop the plate using a 3:2 mixture of hexane and acetone. Your unknown may contain two or three of the standard compounds. Draw a replica (to scale) of this plate in your Results section. Calculate the  $R_f$  values and record in a table (See sample on the next page).

Unknown Colored Compound	
Compound	$R_f$ (s)
1.	
2.	
3.	
4.	
5. Unknown	

From your results, choose the standards that appear to make up your unknown. Next, spot a plate with the standards that you have chosen, your unknown, a mixture of the standards prepared by spotting them one over the other, and a mixture of unknown and the selected standards. Develop the plate and draw a replica of it in your Results section. When you are sure of the identities of the compounds in your unknown mixture, calculate  $R_f$  values for all spots on the last plate. Record your values in a table similar to the one above.

Note: To obtain unequivocal results you may require more plates than those described above. If so, record information only for those plates that run properly and contribute to your determination of the identity of the components of your unknown mixture. Some compounds may streak when run on TLC plates. If they do so consistently, this can be used to help identify the compounds. Use an  $I_2$  chamber to visualize any questionable faint spots.

## 2. TLC Analysis of Analgesic Drugs

Analgesics, compounds that relieve pain, range from aspirin to morphine and related narcotics. In addition to aspirin, several other chemically similar compounds are widely used in non-prescription analgesic tablets. Among these are salicylamide and acetaminophen. Caffeine is sometimes added to these formulations to overcome drowsiness. Other ingredients may be added for different therapeutic effects, such as antispasmodic or slight sedative actions. In addition to the active ingredients, the tablets contain other substances which act as binders or enhance dissolution. In this experiment, you will obtain as an unknown an over-the-counter analgesic drug. You will identify the unknown drug by TLC comparison with these known reference compounds: aspirin (acetylsalicylic acid), acetaminophen (4'-acetamidophenol or 4'-hydroxyacetanilide), caffeine and salicylamide. Your unknown will be one of those listed below; the structures of the ingredients follow.

### Drug

Anacin

Aspirin

BC Original Formula

Excedrin

Tylenol

### Ingredients

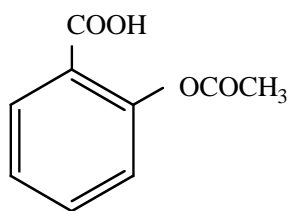
Aspirin and caffeine

Aspirin

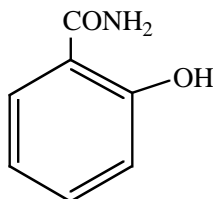
Aspirin, salicylamide, and caffeine

Acetaminophen, aspirin, and caffeine

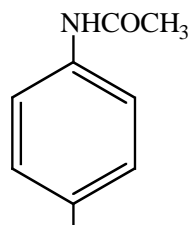
Acetaminophen



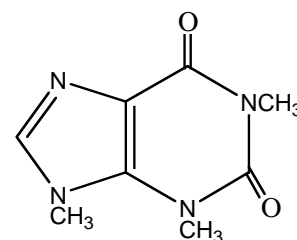
Aspirin



Salicylamide



Acetaminophen



Caffeine

Obtain your unknown analgesic drug and samples (a few drops) of solutions of the four reference compounds. Record the number of your unknown. Dissolve the analgesic in 1 mL of 1:1 CH<sub>2</sub>Cl<sub>2</sub>: methanol. Stir the mixture well and be sure all solid dissolves. Identify your unknown by TLC vs. four samples of the known ingredients, using the same method as you did with your first unknown. Be sure to determine R<sub>f</sub>s of all compounds involved. With this sample, develop the plates using a solvent mixture having the composition 15:1 CH<sub>2</sub>Cl<sub>2</sub>: acetic acid. Observe the developed plates under ultraviolet (UV) light in the hood. Then, develop the plates in an iodine chamber, as demonstrated. Draw replicas of useful plates in your Observations section. Calculate R<sub>f</sub> values of all spots on the first plate and on your confirmatory plate and record the results in tabular format, as you did with the colored compounds.

## B. QUESTIONS

1. An unknown compound, X, was analyzed by TLC using two different eluting solvent mixtures. Three standard compounds, G, H, and I, were used. The R<sub>f</sub> values for the analyses are given below. Which of the three standards is most likely to be compound X? Explain your reasoning in two short sentences.

### 5:1 Hexane: Acetone

<u>Cpd.</u>	<u>R<sub>f</sub></u>
G	0.35
H	0.65
I	0.65
X	0.65

### 3:1 Hexane: Ethyl acetate

<u>Cpd.</u>	<u>R<sub>f</sub></u>
G	0.55
H	0.95
I	0.85
X	0.95

2. An unknown mixture was analyzed using TLC ped on a silica gel plate and developed in 1:1 acetone:hexane, and gave 2 spots with  $R_f$  values of 1.0 and 0.0 respectively.
  - a. Can you say for sure how many compounds are contained in the mixture? If so, how many are there? Explain.
  - b. If not, what further experiments might be performed to determine the number of components in the mixture? Explain.
3. On the basis of the results of your TLC analysis, list the analgesic standard compounds and colored standard compounds in order of decreasing polarity. Explain your reasoning. Keep it brief.

## **F. DISCUSSION AND CONCLUSIONS**

## Notes