CHEM 2219: Exp. #1 Thin Layer Chromatography (TLC)

Objective: In this experiment you will learn to separate the components of a solution using thin layer chromatography; and, to determine the solvent polarity effects. Retention factors will be calculated to determine the identity of the unknown compounds and theoretical plates will be calculated to determine the efficiency of the separation.*

* Chromatography is a useful method for separating components of a mixture of compounds based on their polarity. Thin layer chromatography is especially useful for determining the number of components in a mixture, the identity of the compounds, and the purity of a compound.

Reading Assignment:

MTOL, pp. 125-128 (TLC); and OCLT, pp. 83-108 (chromatography generalities & TLC), pp. 368-369 (TLC technique summary).
Also on **CANVAS** read and answer prelab questions for **– Thin Layer Chromatography**

Concepts:

Capillary Action, Capillary Tube, Development, Fluorescence, Mobile Phase, Polarity, Retention Factor, Solvent Front, Spotting, Stationary Phase, Theoretical Plates, Thin Layer Chromatography (TLC), TLC plate (TLC strip), Visualization

Chemicals:

Acetone, Benzhydrol, Benzophenone, Biphenyl, Dichloromethane, Ethyl Acetate, Hexane, Methanol and Toluene

Safety Precautions:

Wear chemical splash-proof goggles and appropriate attire at all times.
Acetone, dichloromethane, ethyl acetate, hexane, methanol and toluene are flammable liquids.

Materials: ***(For a group of 2)***

6 beakers, 7 pieces of filter paper, 6 labels (med), 6 watchglasses
3 disposable vials for known compounds, 3 labels (small), 5 capillary tubes,
14 fluorescent silica gel TLC plates, 2 disposable vials with unknowns (*1 for each person*),
2 6" rulers and a UV light

References:

https://www.chem.ucla.edu/~bacher/General/30BL/tips/TLC1.html

Background:

Thin-layer chromatography (TLC) is a useful technique for identifying compounds, determining their purity and following the progress of a reaction. One of the main advantages of TLC is that it only requires small quantities of the compound (~ng). It is also much faster than column chromatography. In addition to this, it quickly and easily allows one to determine the optimal solvent system for a given separation problem. In this experiment, the best two of six solvents (acetone, dichloromethane, ethyl acetate, hexane, methanol and toluene) will be determined based on the vertical separation of the spots for the known compounds (benzhydrol, benzophenone and biphenyl).

In the column chromatography experiment, the stationary phase was formed by making a slurry of alumina and packing the column. In TLC, the **stationary phase** is a finely ground matrix of silica gel, fluorescent powder and a binder coated on a plastic film called a **TLC plate** or **TLC strip**. These can be prepared in lab, but are generally purchased already prepared.

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The TLC plates are rather delicate and several precautions need to be taken when working with them. 1.) The TLC strips needs to be kept as dry as possible, so that the coating remains stuck to the film; 2.) A dull pencil should be used when drawing on the TLC plate in order not to scratching through the coating to the film; and, 3.) Care needs to be taken to handle the TLC plates only on the film side. Oils from fingerprints can be transferred to the coating and be mistaken for spots of the components of the mixture.

The **mobile phase,** as in column chromatography**,** is the solvent used. However, unlike column chromatography where the solvent is added to the top of the column and drained down through the stationary phase to move the samples, the TLC plate is developed by adding it to a development chamber and allowing the solvent to move the spots up the plate via **capillary action**.

The TLC plate is marked with a starting line. Using a **capillary tube** (a thin glass tube), a small spot of each of the known dilute solutions is dotted evenly spaced along the starting line. (See Figure 1.) This process is called "**spotting**." In this case, the **development chamber** consists of a beaker, a piece of filter paper, the given solvent and a watch glass cover. The solvent level in the chamber needs to be below the starting line of the TLC plate in order to ensure that the spots travel up the plate rather than dissolving.

During the development, non-polar solvents will cause non-polar compounds to move faster to the top of the plate than polar compounds. This is because the non-polar compounds will dissolve more readily in the non-polar solvents and will interact less with the polar stationary phase. Once the **solvent front** (the level of the solvent on the TLC plate) is 1-2cm from the top, the TLC plate should be removed from the chamber and a line drawn along the solvent front immediately before the solvent evaporates.

Once the solvent has completely evaporated, the spots will be **visualized** by viewing them under a UV light. The spots will appear dark against the fluorescent background. The spots should be outlined using a dull pencil.

If the spots appear in a horizontal row or clumped together, then separation has not been achieved. The spots should have different distances vertically from the start line to the solvent front in order to have good separation. The **retention factor (R_f)** is the ratio of the distance the spot moved from the starting line to the distance the solvent front moved above the origin. The R_f value will be a unitless number between 0.0 and 1.0. The R_f value is consistent to a certain degree for a given compound using the same solvent and the same type of TLC plate. Thus, one can compare the R_f values for the components of the mixture to the R_f values for the known compounds and easily identify the components by matching the R_f values. The number of **theoretical plates, N**, will also be determined in order to verify the efficiency of the separation. The higher the number of theoretical plates the more efficient the separation. (See Figure 2.)

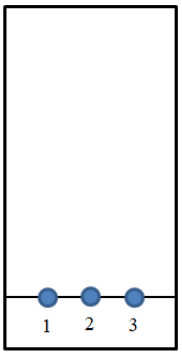

Figure 1: Spots on TLC plate.

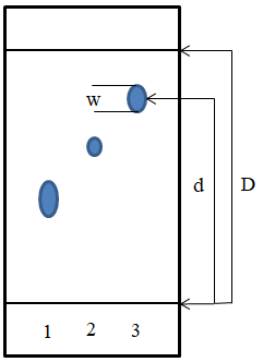

Figure 2: For the calculations, **d, D** and **w** are measured vertically, where **d** is from the starting line to the center of the spot being measured, **D** is from the starting line to the solvent front and **w** is the width of the spot from top to bottom.

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Thin Layer Chromatography Procedure

You are provided with solutions of the three known solids: benzhydrol, benzophenone and biphenyl; and the six solvents: acetone, dichloromethane (CH_2Cl_2 is also known as methylene chloride), ethyl acetate, hexane, methanol and toluene. Your task is to first determine which 2 solvents give the best separation of the compounds. Next you are provided with an unknown solution which contains two of the three known solids: benzhydrol, benzophenone and biphenyl. Your task is to separate and determine the identity of the two compounds using the solvent you determined was the best for separation. Calculate the R_f and Theoretical Plates for all 3 known compounds for the two best solvents and for the 2 spots for the unknown in the best solvent. Tabulate your results and in your conclusion make comments about your decisions.

Preparation of Developing Chambers*:

***Note:** Two students will share developing chambers, but **each student should develop their own set of TLC plates and a different unknown**. The TLC plates for both students may be run simultaneously.

1. Obtain six labeled plastic beakers, 6 pieces of filter paper and 6 watch glasses to be used as developing tanks for the different solvents that will be used: **acetone, CH_2Cl_2, ethyl acetate, hexane, methanol and toluene.**

2. Line the inside of each beaker with a piece of large filter paper (curved against the wall of the beaker).

3. Take the beakers to the hood and add about 10 ml (or a depth of 6mm or ¼") for each solvent to the appropriate beaker. (*Rulers may be found in your drawer*.) Return to your lab bench. Slosh solvent on the filter paper so that the vapors can saturate the air inside the beaker. Cover the beakers with a large watch glass, then prepare the TLC plates.

Note: Keep these beakers covered as much as possible in order to keep the chambers saturated with solvent vapors and to prevent the inhalation of hazardous or smelly chemicals.

TLC of Reference Compounds and Unknown Mixture:

Note: TLC plates containing spots of each of the three compounds will be tested in each of the six solvents to determine the best solvent for separation.

Each student will develop their own plates and their own unknown.

1. Obtain six of the fluorescent silica gel TLC plates from the desiccator.* Using your ruler and a dull #2 pencil (NOT a pen) lightly draw a line 1.5cm (~ ½") parallel to the bottom of the strip on the "chalky side". Along the line make 3 tick marks perpendicular to the line and evenly spaced across the line. (See Figure 1.)

***Be careful.** Do not touch the surface of the strips with your fingers.

2. Obtain 3 disposable vials with caps and labels. Label vials: Benzhydrol, Benzophenone and Biphenyl. Add 6 mm or ~¼" of known solutions to their corresponding vials. Cap vials and place in a small beaker so that they do not tip over.

3. Obtain 5 capillary tubes: 3 for known compounds and 1 for each partner's unknown mixture.

Note: To practice spotting, obtain a piece of filter paper. Before spotting your TLC plate practice making small spots (<1 mm) on the filter paper. Try to keep the spots as small as possible. If the spots are too large, you may need to obtain a new/different capillary tube. If the spots are too small, though, then several applications of the compound may be needed to get an adequate concentration of the compound in the spot.

4. Using a separate capillary for each compound, apply spots of the known solutions of benzhydrol, benzophenone and biphenyl to the line on each of the six plates.

Note: You should keep the compounds in the same order for all six plates in order to avoid confusion.

5. Slide the TLC plates onto a solid surface like a notebook and take them to a UV (black light) viewing box. Verify that the spots are visible on each of the tick marks on each of the TLC plates prior to development to ensure that you have applied enough material. If not all spots are visible add more of the given compound and view the TLC plate again. If the spots overlap, discard the TLC plate and make a new one.

Caution: **Do *not* look directly at the UV source lamp! It can damage your retina.**

6. Hold the TLC plate up next to the outside of the beaker to verify that the level of the solvent in the beaker is below the line containing the spots (i.e., less than 12 mm or ½").

Note: If the solvent height is higher than the spotted line on the TLC plate, the experiment will not work. If this is the case, pour a small amount of the solvent from the beaker into the waste container and recheck solvent height. If too much solvent is poured out, it will need to be replaced. If there is not enough solvent in the beaker, the solvent front will not be able to move up to within 12mm or ½" of the top of the TLC plate.

7. Place the TLC plates in the solvent beakers.* Once the TLC plates are properly placed, cover each beaker with a watch glass.

***Note:** Make sure the TLC plates do not touch each other or the filter paper. Be very careful not to slosh any of the solvent onto the TLC plates.

8. Allow the solvent to rise within 12 mm (~ ½ ") of the top of the TLC plate. The amount of time required will vary with the solvent and you should record the order (of the solvents) in which the plates were removed.

9. Remove the TLC plates and immediately mark the location of the solvent front lightly with a pencil. Then write the name of the given solvent on the TLC plate above the solvent front line.

Note: The hair dryer from your common drawer may be used to speed up evaporation of the solvent. There are also hair dryers in the hoods on either end of the lab, if your common drawer does not have one in it. Be sure to secure the TLC plates before applying the air. Otherwise when the air from the hair dryer is applied, the plates might fly into the sink or onto the floor and become contaminated.

10. Once the TLC plates have dried*, take them to the UV (*black light*) viewing box. While viewing the TLC plates under the black light, outline the spots on each plate lightly with a pencil.

> ***Note:** If the plate has an overall dark appearance under the UV light, then the solvent has not completely evaporated. Use a hair dryer to dry the plate for a few minutes and view the plate again. If you are still having difficulty seeing the spots, consult with your instructor.

11. Determine and record the two best solvents by figuring out which two solvents give the best / widest separation of the spots in the vertical direction. Choose one of the two solvents as the "best" solvent to use for the unknown. Record your choice and explain why you chose it.

12. Obtain an unknown sample from the hood. Record your unknown# and run your unknown using the best solvent only. Develop and view the unknown TLC plate the same as before. (***Note:** You only have to perform TLC on your unknown. Your partner only has to perform TLC on their unknown.*)

13. While the unknown TLC strip is in the development chamber, there is time to measure the values **(d, D & w)** needed later for the calculation of the retention factor, $\mathbf{R_f}$, and the approximate number of theoretical plates, **N**, for each compound for the two best solvent systems. Record all values in cm to the nearest mm ($^1/_{10}$ th cm). (See Figure 2.) *If you have a ruler at home, you may finish this step after class.*

$\mathbf{R_f = d/D}$ where **D** is the distance from the starting line to the solvent front and **d** is the distance from the starting line to the center of the spot.

$\mathbf{N=16(d/w)^2}$ where **d** is again the distance from the starting line to the center of the spot and **w** is the width of the spots (or the diameter of the spot measured perpendicular to the starting line).

14. Once the unknown TLC plate has been visualized, verify your findings with a TA or an instructor.

15. Using 2" wide clear packing tape from the cart, attach your TLC plates to the back of the duplicate page of your prelab table in your lab book. Be sure to cover the TLC plate completely with the tape, so that the silica gel coating does not inadvertently get scratched.

16. Dispose of chemicals appropriately. Clean and return all glassware & equipment to their original locations.

Post Lab:

1. Calculate the R_f and theoretical plates for all three compounds in the two best solvents. (These will be your standard values.)
2. Calculate the R_f and theoretical plates for the two compounds in the unknown.
3. Determine which two compounds are present in your unknown by matching R_f values with the standards in the same solvent.
4. Tabulate your results for R_f and N for the two best solvents and for your unknown.
5. In your conclusion, list your unknown number and your identification of the 2 compounds in your unknown; explain how you made your determination; and discuss the efficiency of the separation based on the theoretical plates calculated. Also discuss whether the experiment was successfully performed and / or any problems that arose during the experiment and your recommendation for how to avoid those problems if you were to redo the experiment.