

RECITATION NOTES FOR EXPERIMENT # 5 A&B

THIN LAYER CHROMATOGRAPHY

Have your lab textbook available for quick reference to specific pages, indicated in red.

BASIC PRINCIPLES OF CHROMATOGRAPHY

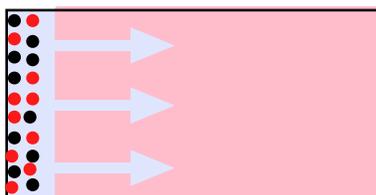
Chromatography is yet another technique for the analysis and separation of chemical mixtures. The technique is based on a polarity interplay between the sample and two other substances called the **solid (or stationary) phase**, and the **mobile phase**, which can be a liquid or a gas. As their names indicate, the stationary phase does not move, whereas the mobile phase flows across, or through the solid phase.

In this “love triangle,” the sample to be separated first becomes **adsorbed** onto the surface of the solid phase through polar interactions. More polar samples adhere (or bind) more strongly than less polar ones. The mobile phase then comes in and sweeps across the stationary phase, competing for the sample. If the mobile phase is a solvent, it is called the **eluting solvent**. The more polar the solvent, the greater its capacity to carry the components of the mixture with it, “yanking” them away from the solid phase as it moves. Different components with different polarities will travel at different rates as the solvent moves, causing their separation.



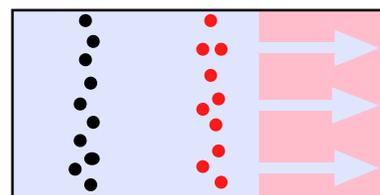
1. Initial state: Sample is adsorbed on the solid phase.

- sample mixture
- stationary phase, or solid support



2. Eluting solvent begins to flow through the solid phase.

- eluting solvent



3. As the solvent moves, the components of the mixture move at different rates.

- the less polar component travels faster.
- the more polar component travels slower.

Some of the most common solid adsorbents used as stationary phases in chromatography are listed in [table 19.1, p. 760](#). In the organic chemistry lab the most commonly used are **silica gel** and **alumina** due to their versatile polarity range. Likewise, some common eluting solvents are listed in [table 19.2, p. 761](#) by order of increasing polarity, and therefore increasing eluting power. **Methylene chloride, ethyl acetate, and ethanol** are among the most commonly used.

[Table 19.3, p. 762](#), lists some functional groups by order of elution, meaning how fast they move in a chromatographic separation. Again, the more polar ones (alcohols, amines, and acids) move slower, whereas the less polar ones (hydrocarbons and ethers) move faster.

THIN LAYER CHROMATOGRAPHY (TLC)

In thin layer chromatography the solid phase (silica gel or alumina) is applied as a thin coating on a plastic sheet or glass slide, called a **TLC plate** ([fig. 20.4, p. 783](#)). Using a capillary tube, a solution of the sample is applied on the solid support as a spot, a technique known as **spotting**. When the sample dries it becomes adsorbed onto the solid support. This process can be repeated several times to produce a more concentrated spot.

SPOTTING TECHNIQUE

1. Throughout these operations, keep in mind that the coating on the TLC plate is very fragile and easily chips. Plates must be handled with care.
2. Using a **pencil, not ink**, draw a horizontal line **about 1 cm from the bottom of the TLC plate** to mark the starting point of the sample for elution. Do not apply too much pressure, or you will break the coating. The sample spots are to be applied on this horizontal line.
3. Dip the tip of a capillary micropipette in a solution of the sample to be analyzed. Capillary action will cause the liquid to rise up the pipette.
4. Lightly touch the solid support with the tip of the micropipette as if using a fountain pen. A small amount of solution will transfer to the plate forming a spot. As with a real pen, the longer you leave the pipette in contact with the plate the bigger the spot. We don't want big spots, therefore just "peck" the coating and withdraw the pipette.
5. Wait until the spot dries before applying the next one, which should be done on exactly the same place. With most solutions, 3 or 4 applications **of the same solution** should suffice to produce a good spot. A spot that's too dense or too big can cause problems during the separation.
6. If you are applying several **different samples** on the same plate, **make sure their spots do not touch each other**. Adjacent spots must be separated by a minimum distance of 0.5 cm, or they'll run into each other during the separation.
7. Likewise, spots must not be too close to the edge of the plate, or they can run off the plate during separation. Again, spots should be separated from the edges by a minimum of 0.5 cm, except for the bottom of the plate, in which case they should be separated by a minimum of 1 cm. The reason is that the plate will be immersed in the eluting solvent to effect the separation, and the eluting solvent should not touch the spots when the separation begins.

THE DEVELOPING CHAMBER

While the spots are being applied and allowed to dry, prepare a **developing chamber** for the separation (that's a fancy name for a beaker or jar that's **slightly taller** than the TLC plate). Refer to **fig. 20.5 on p. 784**.

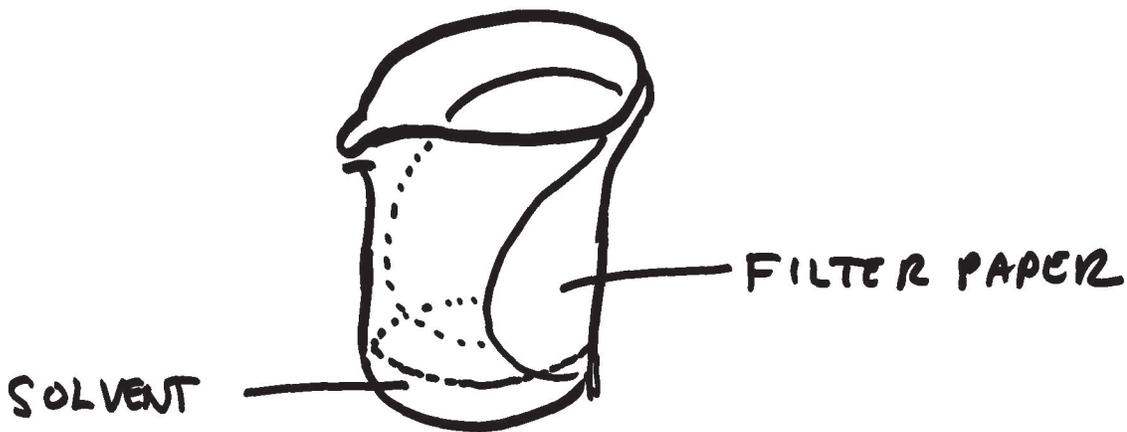
To do this take a clear container and place a small amount of eluting solvent inside, **just about enough to cover the bottom**. You can also take a piece of filter paper whose diameter is about the height of the container and place it around the walls. Squirt some of the eluting solvent on the filter paper to make it wet so that it adheres to the walls better. This keeps the space inside the container saturated with solvent vapors to minimize losses by evaporation and improve the separation. Also have a **lid or watch glass** ready to cover the container during the separation.

ELUTION TECHNIQUE

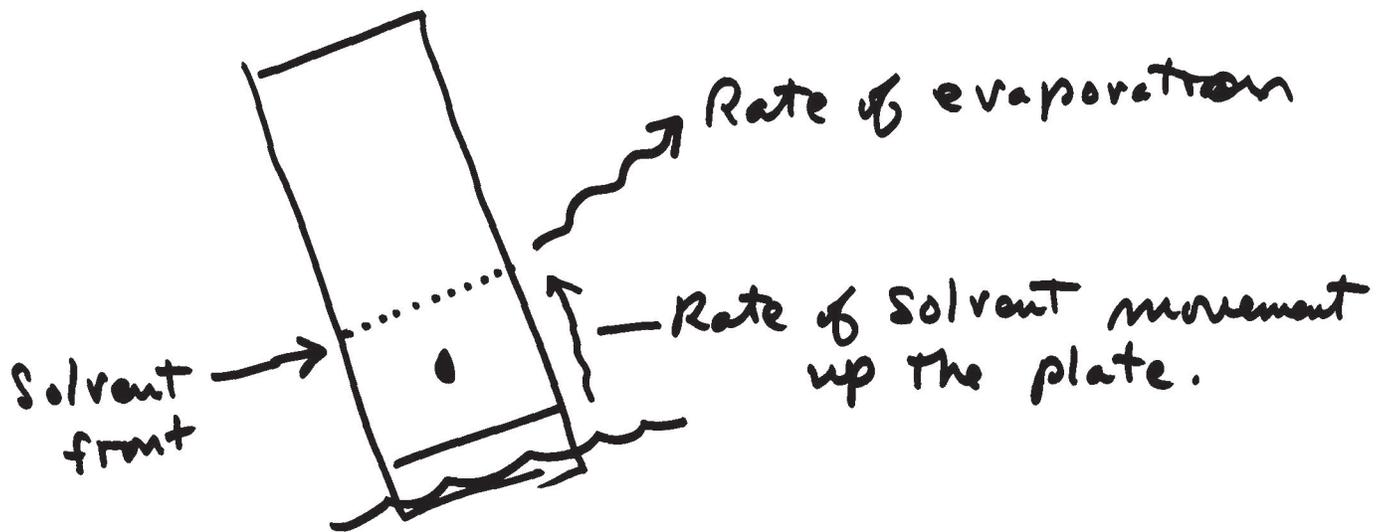
Once the TLC plate has been spotted and ready to go, **carefully** place it inside the developing chamber, trying to avoid splashing. Remember, we don't want the solvent to touch the spots before we start the separation. If it does, it will cause the spots to smear and they'll become useless.

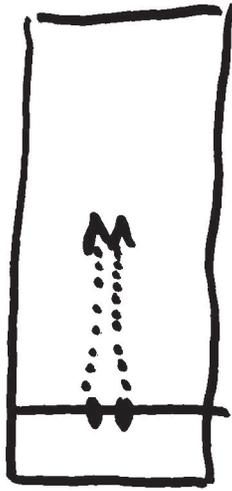
The plate should be placed upright, slightly inclined against the wall of the container. **The solvent should not touch the spots** when the separation first begins, that's why its level should be below the spots by at least 0.5 cm. Cover the container and wait for elution to proceed.

By capillary action, the solvent will immediately start rising up the TLC plate. As soon as it comes into contact with the spots, it will cause the less polar components of the mixture to start moving. The more polar components will also move, but at a less noticeable rate. The following diagram illustrates the process.

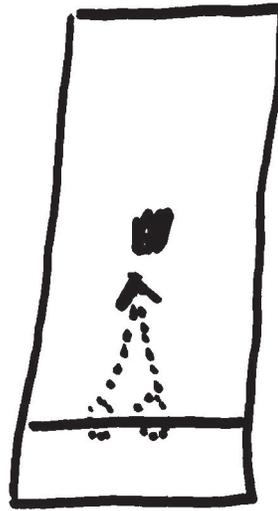


A FILTER PAPER DISK SET AGAINST THE WALLS OF THE DEVELOPING CHAMBER SLOWS DOWN SOLVENT EVAPORATION INSIDE THE CHAMBER AND THEREFORE SPEEDS UP THE SEPARATION

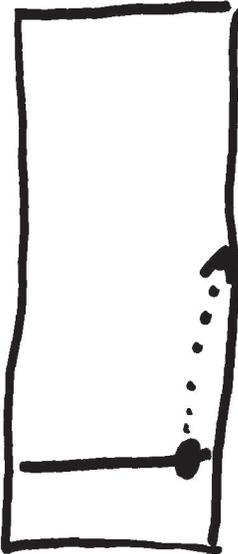




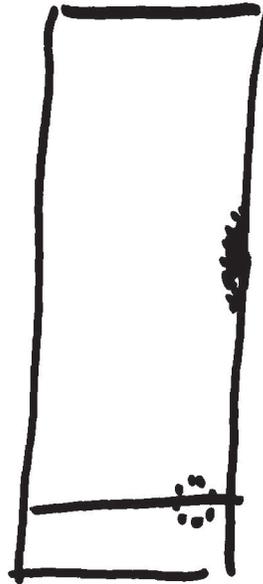
SPOTS TOO CLOSE TOGETHER



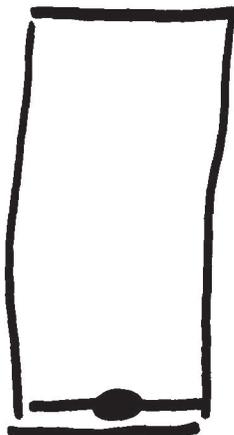
Coalescing



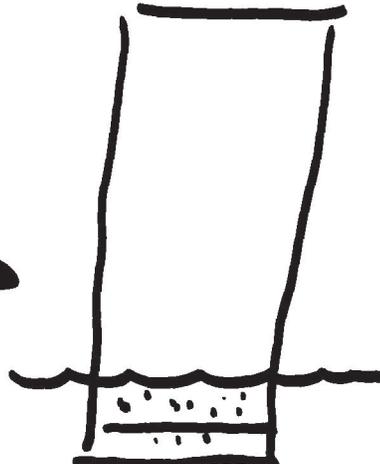
SPOT TOO CLOSE TO THE EDGE



blurred & Tailing



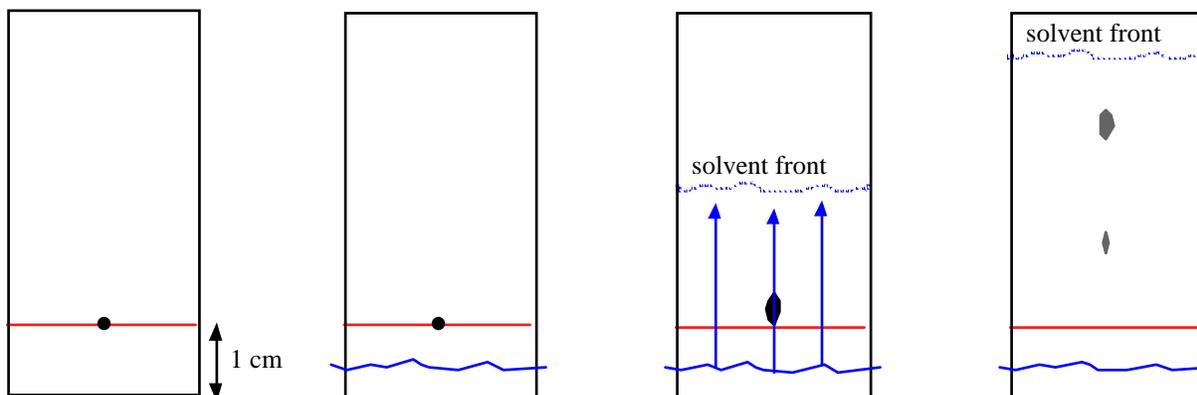
SPOT TOO LOW



DISSOLVES IN SOLVENT

SOLVENT LEVEL

PART A – Separating the components of a mixture using a given solvent.



Initial TLC plate with spot

- pencil line
- initial spot

TLC plate inside the developing chamber

- solvent level

As the solvent moves up, it will cause the spot to move as well

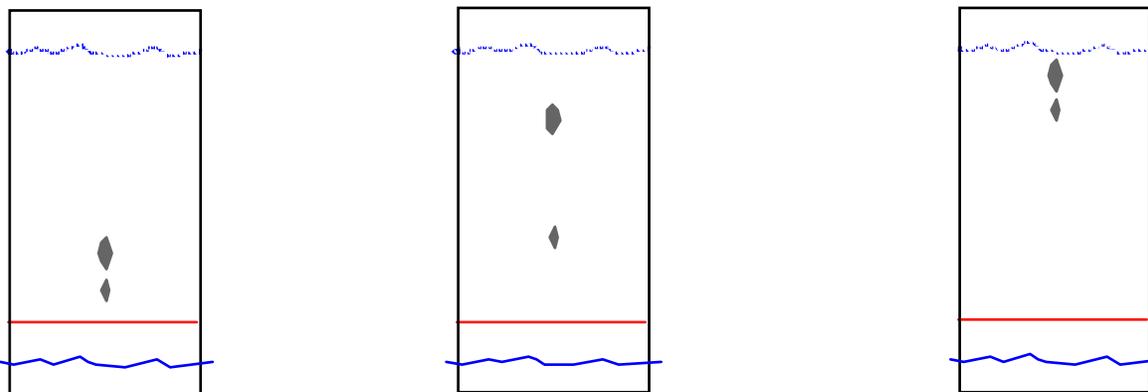
A good solvent causes good separation of the components of the mixture.

When the plate is first placed inside the developing chamber, its bottom should be **as parallel as possible with the solvent level**. If the plate enters the solvent at an angle, it will cause a slanted solvent front line which may result in poor separation and uneven alignment of the spots.

When the solvent front is about 0.5 to 1 cm from reaching the top, it's time to stop the elution. **Do not allow the solvent front to reach the top of the plate**. That may cause erroneous R_f values and may cause spots that are close together to run into each other. Take the plate out with tweezers and **mark the solvent front line with a pencil** as soon as possible. If you wait too long, it will fade and you will miss a crucial part of information needed to calculate the R_f values.

If a poor separation is obtained with a certain solvent, the experiment needs to be repeated with a solvent of different polarity. As with crystallization, solvent mixtures can be used to achieve intermediate degrees of polarity. Low polarity solvents will cause the spots to move more slowly. High polarity solvents will do the opposite. Either extreme can cause poor separation. Only practice will enable you to gain a sense of how to reach a "happy medium."

PART B – Finding the solvent that provides the best separation.



A low polarity solvent may move the spots too slowly for a good separation

A solvent of intermediate polarity produces the best results

A solvent that's too polar may move the spots too fast for a good separation

VISUALIZATION TECHNIQUES

After taking the TLC plate out of the developing chamber and marking the solvent front line, allow the plate to dry. Drying can be accelerated by placing the TLC plate on a **lukewarm** surface. Remember, the plate is made of plastic, **if the plate is too hot the plastic will melt**. Once the plate is dry, you need to be able to see the spots. Unless your sample is colored, this is not always possible with the naked eye. **Visualization techniques** are methods that enable you to see otherwise “invisible” spots. There are several methods available, but the two most commonly used in the organic lab are **iodine staining**, and use of a **UV lamp**.

Iodine reacts with many organic substances to produce compounds which are brownish. Place the TLC plate inside an **iodine chamber** (again, a fancy name for a jar that contains a few iodine crystals). Close the jar, and within a few minutes spots will appear on the plate. This process can be accelerated by placing the chamber on a **warm** surface. Once you can see the spots, take the TLC plate out of the chamber with tweezers or wearing gloves (**iodine is a toxic substance, and so are its vapors**). Mark the spots with a pencil.

Most commercial TLC plates contain not only the solid phase coating, but also a fluorescent substance that is **sensitive to ultraviolet (UV) light**. By shining UV light with a special lamp on the plate, the coating will appear greenish. If there are spots or other substances blocking the light, they will appear blackish. This will enable you to mark the places where the spots are with a pencil.

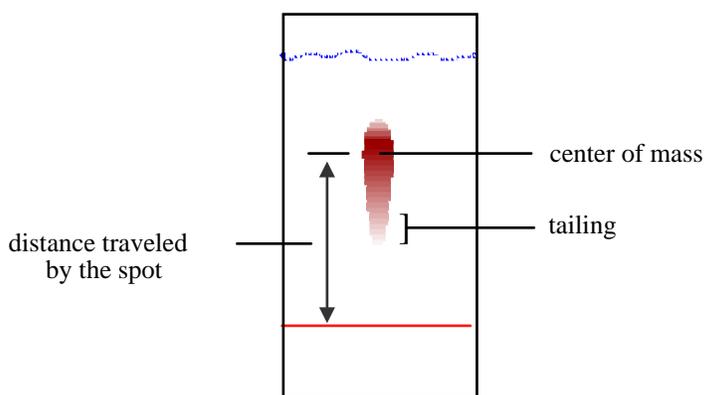
Since iodine vapors are toxic, only the UV lamp will be used in the lab.

CALCULATING R_f VALUES

The scientific measure of the chromatographic separation in TLC is the **R_f value**, variously interpreted in different sources as *retardation factor*, *ratio to the front*, etc. (see p. 788). It is given by the expression

$$R_f = \frac{\text{distance traveled by the spot}}{\text{distance traveled by the solvent front}}$$

See **fig. 20.8 on p. 788** for an example. Some spots are somewhat large, while others “tail.” Tailing tends to occur with ionic or highly polar substances that are reluctant to move because they bind too strongly to the solid phase. When this happens the center of mass of the spot is used to measure the distance traveled by the spot. The reaction with iodine as a visualization method is particularly good at revealing the densest part of the spot.



By definition, **R_f values are always less than 1**. An R_f value of 1 or too close to it means that the spot and the solvent front travel close together and is therefore unreliable. This happens when the eluting solvent is too polar for the sample. Another run must be made using a less polar solvent to improve the separation.

Likewise, if the spot hardly moves and its traveled distance is nearly zero, there has probably been no chance for separation. This happens when the eluting solvent is not polar enough for the sample. Another run must be made using a more polar solvent to improve the separation.

RELATIONSHIP BETWEEN R_f VALUE AND POLARITY OF THE SAMPLE

It was mentioned before that the polarity of the sample and its rate of movement are inversely related. The more polar spot travels slower, and the less polar spot travels faster. R_f values, on the other hand, are directly related to the rate of movement. The fastest moving spot has the highest R_f value.

Therefore R_f values and polarity are inversely related. The spot with the highest R_f value is the least polar (fastest moving), and the spot with the lowest R_f value is the most polar (slowest moving).

USES OF R_f VALUES IN TLC

R_f values are frequently used to compare a known and an unknown substance to determine if they are the same. **As long as their spots are run simultaneously on the same plate**, the argument can be made that if their R_f values are the same, and if the general appearance of the spots is the same, then they are the same compound.

R_f values depend on:

- The polar nature of the solvent used
- Polar nature of the stationary phase
- Polar nature of the sample
- Conditions of the experiment

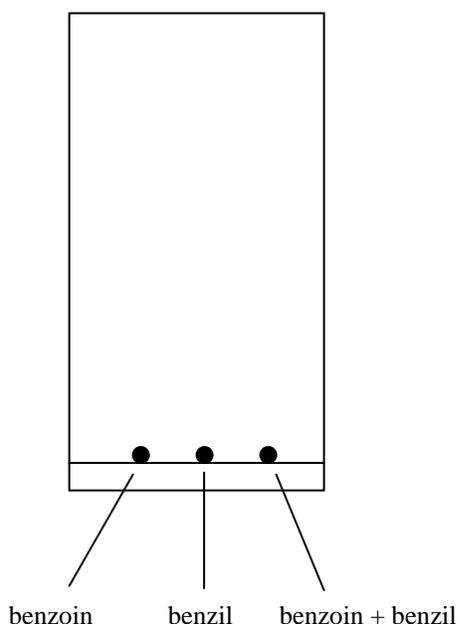
R_f values are therefore not exactly reproducible from one experiment to another, even if an effort is made to carry them under the exact same conditions. **When comparing two or more substances, they must be run simultaneously on the same plate** or the comparison is invalid.

EXPERIMENT 5: INSTRUCTIONS FOR PART B.

Although the lab textbook proposes several compounds and solvents that you can use in part B, we will use only two substances and three solvents. The two substances are benzoin and benzil. The solvents are hexane (nonpolar), methylene chloride (moderate polarity), and acetone (polar). Therefore, you will **spot three solutions onto each of three TLC plates**:

- a. **Benzoin** only solution
- b. **Benzil** only solution
- c. **Benzoin and benzil** solution

1. **PREPARE THREE TLC PLATES.** Spot each plate with the above solutions, as follows:



2. **DEVELOP THE 3 PLATES AS FOLLOWS:**

- Plate 1 in **hexanes**
- Plate 2 in **methylene chloride**
- Plate 3 in **acetone**.

3. **CALCULATE R_f VALUES** for the spots in the three plates.

4. **REPORT RESULTS** explaining which solvent effected the best separation of the benzoin-benzil mixture (third spot), and why, based on polarity of the substances and the solvents used.

The chemical structures of benzoin and benzil are given on **p. 309 of the lab textbook**, under Exp. 36B.