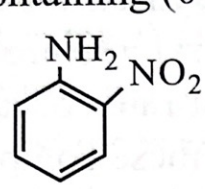
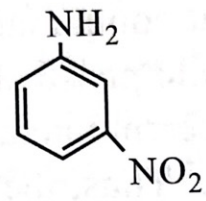
Thin Layer Chromatography (TLC)

The purpose of this experiment is to enable the student to:

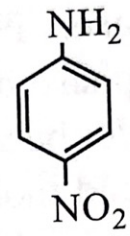
1. Learn about the practical and theoretical aspects involved in thin layer chromatography.
2. Learn how to use TLC as a tool in identifying what is present in an unknown mixture containing (0 -3) of the following compounds:



o-nitroaniline



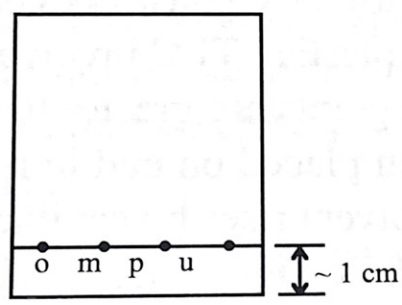
m-nitroaniline



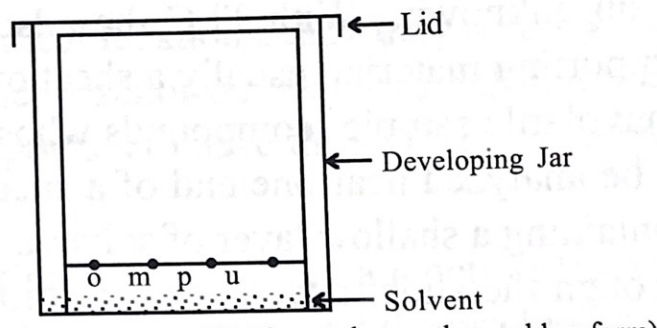
p-nitroaniline

Figure 1. Steps Involved in Thin Layer Chromatography

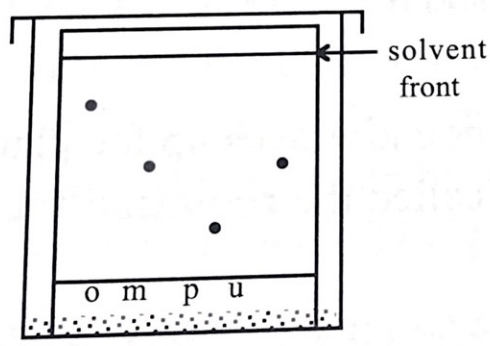
Step 1. Spot Plate



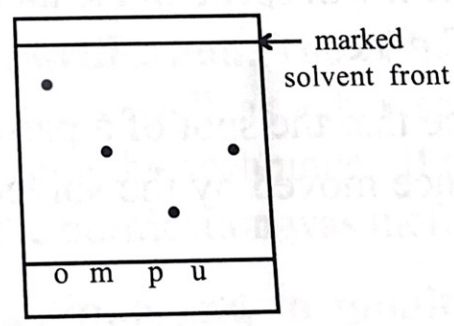
Step 2. Place Plate in Developing Jar



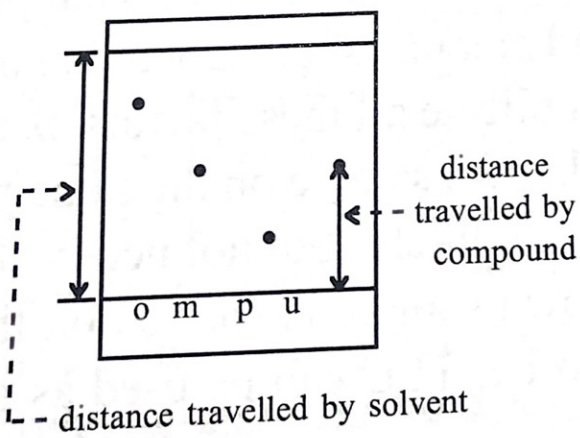
Step 3. Develop Plate



Step 4. Visualize Developed Plate



Step 5. Determine R_f Value for Each Compound



$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

BACKGROUND

The most modern and sophisticated methods for separating mixtures that the organic chemist has available all involve chromatography. **Chromatography** is a general term that refers to a number of related techniques used for analyzing, identifying, or separating mixtures of compounds. All chromatographic techniques have one principle in common; a liquid or gaseous solution of sample, called the **moving phase**, is passed (moved) through an adsorbent, called a **stationary phase**. The different compounds in the sample are moved through the stationary phase by the mobile phase at different rates because of physical differences and/or because of different interactions of these compounds with both the stationary and mobile phase. Thus, the individual components in a sample become separated from each other as they pass through the stationary phase.

Thin layer chromatography (TLC) is a sensitive, fast, simple, and inexpensive analytical technique that can be used as a means to help identify what is present in an unknown. With TLC the adsorbent (stationary phase) is coated onto a supporting material, usually a sheet of glass or plastic. TLC involves spotting a nonvolatile sample (compounds whose boiling points are greater than ~ 130°C) to be analyzed near one end of a sheet and then placed on end in a covered jar containing a shallow layer of solvent. As the solvent rises by capillary action up through the slide, differential partitioning of the components in the mixture is occurring between the moving solvent and the stationary adsorbent. The more strongly a given component of the mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the TLC plate.

The distance that the spot of a particular compound moves up the plate relative to the distance moved by the solvent front is called the **retention factor**, or $\mathbf{R_f}$ value.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent}}$$

The R_f value for a compound is a constant, only if variables such as temperature, solvent, adsorbent, thickness of adsorbent, and amount of compound on the plate are held constant. Because it is difficult to duplicate all these factors exactly, an unknown sample is usually compared with a known sample on the same plate. If two substances have the same R_f value, they are likely (but not necessarily) the same compound. Thus, as long as an unknown sample is spotted on the same plate as the possible compounds that it could be, TLC can be used as a tool to

help determine the identity of an unknown sample - the two substances having the same R_f value are likely the same compound.

TLC has many common uses in the organic chemistry laboratory. It's many uses are briefly discussed below:

1. **To determine the number of components in a mixture.** Knowing the number of components in a mixture (such as a crude reaction mixture or some plant extract) aids in planning further analytical and separation steps. However, there is no guarantee that a single spot is a single substance; two or more substances may not be separated by this technique and therefore, show up as just one spot. In order to substantiate that one spot is a single substance, different chromatographic conditions (adsorbents & solvents) need to be tried; if under these new conditions there is only one spot, then you are almost guaranteed that one spot is just one substance.

2. **To determine the identity of two substances.** If two substances spotted on the same TLC plate give spots in identical locations, they may be identical; if they are not in the same location, they cannot be the same. It is possible, however, for two closely related compounds to have the same position on the TLC plate.

3. **To monitor the progress of a reaction.** By sampling a reaction from time to time it is possible to watch the reactants disappear and the products appear using this technique. Thus valuable time is saved, by knowing the optimum time in which a reaction can be halted.

4. **To determine the effectiveness of a purification.** The effectiveness of a distillation, recrystallization, extraction, as well as other separation and purification methods can be monitored using this technique. If more than one stop is obtained on the TLC plate, then the purification was ineffective.

5. **To determine the appropriate conditions and to monitor a column chromatographic separation.** TLC is generally unsatisfactory for purifying and isolating large quantities of material. For large quantities of material, column chromatography (a variation of TLC) is the method of choice for separation and purification. In column chromatography, the stationary phase (adsorbent) is packed in a vertical glass column. The sample is added to the top of the column; then solvent is carefully poured through the column to wash the components of the sample, one by one (ideally), down the adsorbent to the outlet. The correct adsorbent and solvent used to carry out column chromatography can be determined rapidly by TLC.

The two most common coatings (adsorbents) for TLC are **alumina**, Al_2O_3, and **silica gel**, SiO_2; both of which are polar. Of the two, alumina when anhydrous, is more active, i.e., it will adsorb polar substances more strongly. The strength of interaction varies among compounds. One can use the following rule of thumb: the more polar the functional group, the stronger the bond to alumina or silica gel. Alumina is the adsorbent of choice for the separation of nonpolar substances such as hydrocarbons, alkyl halides, and ethers; whereas, silica gel is the adsorbent of choice for the separation of polar substances; such as aldehydes, ketones, alcohols, amines, and carboxylic acids. In an extreme situation very polar substances on alumina do not migrate from the starting point ($R_f = 0$), and nonpolar substances travel with the solvent front ($R_f = 1$) if chromatographed with silica gel. These extremes of behavior are markedly affected, however, by the solvents used to carry out the TLC. For example, an amine adsorbed on alumina might not migrate with hexane, however it will migrate with ethyl acetate. A polar solvent will carry along with it polar substrates, and nonpolar solvents will do the same with nonpolar compounds.

The following table lists common solvents used in chromatography. In general these solvents are characterized by having low boiling points and low viscosities that allow them to migrate rapidly. The chromatographic solvent of choice is one in which the components of the mixture dissolve and thus are able to interact with the mobile phase and thereby move off the origin. Hence the general rule of thumb "like dissolves like" should be used.

Chromatographic Solvents (Listed in order of increasing polarity)

1. Alkanes (petroleum ether, ligroin, hexane, pentane)
2. Benzene
3. Alkyl halides (dichloromethane, chloroform)
4. Diethyl Ether
5. Ethyl Acetate
6. Acetone
7. Alcohols (methanol, ethanol)
8. Water
9. Acetic Acid

The order in which solutes migrate on TLC is the same as the order of solvent polarity. The largest R_f values are shown by the least polar solutes. The following table gives the order of solute migration for various functional groups (which is also arranged in order of increasing compound polarity and order of decreasing R_f value when a polar adsorbent is used) on TLC.

Order of Migration (Listed in order of decreasing Rf value)

1. Alkanes
2. Alkyl halides
3. Alkenes
4. Aromatic hydrocarbons
5. Aromatic halides
6. Ethers
7. Esters
8. Ketones and Aldehydes
9. Amines
10. Alcohols
11. Phenols
12. Carboxylic acids

Visualization of the spots of a developed TLC plate can be accomplished by the following common techniques. The last two techniques are used to "visualize" colorless compounds.

1. **Direct Visualization.** If a compound is colored, it can be visualized directly. Compounds that posses a color absorb light in the visible portion of the electromagnetic spectrum, which ranges from about 400 nm (violet light) to about 800 nm (red light). Hydrocarbons will have a color if they are extensively conjugated (i.e., alternating double-single bonds are present). An extensively conjugated hydrocarbon is one that has 9 or more conjugated bonds. The compounds used in this experiment (i.e., o-, m-, and p-nitroaniline) absorb visible light (i.e., they are yellow) and thus direct visualization is used. Though nitroanilines have only 4 conjugated bonds they have a color because the nitro group is a powerful chromophore (i.e., compound having unsaturated π orbitals).

2. **Ultraviolet Light.** Organic compounds capable of absorbing UV light, will show up as dark spots. Wavelengths of light from about 200 - 400 nm constitute the ultraviolet region of the electromagnetic spectrum. Absorption of UV light is restricted to organic functional groups containing chromophores. Conjugation of chromophores leads to absorption of light further into longer wavelengths of light. The compounds used in this experiment (i.e., o-, m-, and p-nitroaniline) also absorb UV light because they contain chromophores (i.e., they are unsaturated).

3. **I_2 vapors.** Iodine vapor will preferentially be adsorbed by substances on the plate and they will appear as brown spots. The plate is removed from the jar and the outline of the spots traced lightly in pencil because the iodine will eventually sublime. If the plate is left in the iodine vapor too long, the entire plate will become dark, and the spots will no longer be distinguishable. In such cases, the plate is removed from the iodine vapor and observed carefully as the iodine sublimes. The spots should then become apparent, and then circled with a pencil. The use of iodine vapors as a visualization technique is quite useful to visualize most organic compounds, with the notable exception of alkanes and alkyl halides. Because nitroanilines (the compounds used in this experiment) are neither alkanes or alkyl halides, I_2 vapors can be used to visualize them.

EXPERIMENTAL PROCEDURE

Commercially bought Eastman Kodak silica gel 20 x 20 cm TLC sheets with fluorescent indicator (No. 13181) are used in this experiment. The sheets have been cut to an approximate size of 4 x 6 cm. These sheets consist of a 100-micron-thick coating of silica gel on an inert, flexible poly(ethylene terephthalate) support. Polyacrylic acid has been added as a binder. The fluorescent indicator added to the silica gel when observed under a 254-nm ultraviolet light, will spot compounds that quench or enhance fluorescence.

Step 1. Carefully spot the TLC plate, using a microcapillary pipet, with: a) o-nitroaniline, b) m-nitroaniline, c) p-nitroaniline, and d) unknown. All compounds must be spotted on the same line about 1 cm up the plate. Make the spots as small as possible (about 2 - 4 mm in diameter) and that each spot is separated by a distance of about 8 - 10 mm. (Samples that are spotted too close together tend to spread out and run together as they are developed.)

Step 2. Prepare the developing jar by adding about 5 mL of solvent (50 : 50 petroleum ether : chloroform). Prop the plate upright in the center of the jar (spots at the bottom) and cap the jar. **Do not move the jar during the development.** (The solvent must be below the level where the compounds were spotted; if the spots are below the solvent level, they will be dissolved away by the solvent.)

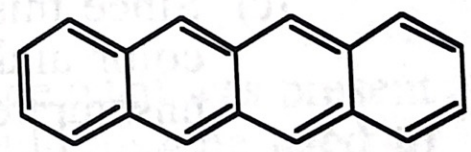
Step 3. Let the plate develop by allowing the solvent to rise up the plate by capillary action. The properly chosen adsorbent (silica gel) and solvent (50 : 50 pet. ether : chloroform) allows for the separation of the nitroanilines. When the solvent has risen almost to the top of the plate, open the jar, carefully remove the plate, and quickly mark a line across the plate at the solvent front with a pencil before the solvent evaporates. (Do not allow the TLC plate to remain in the developing chamber after the solvent has reached the top; spots allowed to stand on a completely moistened plate on which the solvent is not in motion expand by diffusion.)

Step 4. Visualize the spots. Since all the nitroanilines are yellow, they can be directly visualized with your eyes. Outline each spot with a pencil, for the spots may disappear with time. [With all the nitroanilines all three methods of visualization, previously discussed, can be used.]

Step 5. Determine what is present in your unknown and calculate the R_f value for each compound. If the spot is elongated, the "center" is estimated (usually closer to the leading edge). This "tailing" is sometimes caused by too much sample in the original spot. [NOTE: In the example shown in Figure 1, only m-nitroaniline was present in the unknown.]

PROBLEMS

1. What would you expect to observe after plate development and visualization as a result of the following errors in the use of TLC:
   (a) the solvent level in the developing chamber is higher than the spotted sample.
   (b) too much sample is applied to the TLC plate.
   (c) the TLC plate is allowed to remain in the developing chamber after the solvent level has reached the top.
   (d) samples that are spotted too close together.
2. Calculate the R_f value for a compound that runs up 5 cm and whose solvent front is 20 cm.
3. If two compounds have R_f values of 0.50 and 0.61, how far will they be separated from each other on a plate when the solvent is developed to (a) 5 cm and (b) 20 cm?
4. Arrange the following compounds in order of increasing R_f value if silica gel is used as an adsorbent and hexane is used as the solvent:
   $CH_3(CH_2)_3COOH$, $CH_3(CH_2)_{10}CH_3$, $CH_3(CH_2)_6OH$
5. What will be the appearance of a TLC plate if a solvent of too low a polarity is used for the development of a very polar compound?
6. List some of the common uses of TLC in the organic chemistry lab.
7. Why is TLC only useful for nonvolatile samples?
8. Explain if each of the following visualization techniques could or could not be used in the visualization of the following compounds - (a) direct visualization, (b) iodine vapor, or (c) UV light:

   (a) $CH_3(CH_2)_8Br$ (b) cyclohexene (bp = 80°C) (c) 
9. What does an R_f value of 0 mean?
10. What can you conclude from a student who reported an R_f value of 1.2?
11. An unknown has two possible compounds. Under the conditions run for the knowns, the R_f values were found to be 0.62 and 0.65, respectively. Could a positive identification be made if the solvent is developed to 5 cm? Explain.
12. An unknown containing 2-bromooctane and 2-decene are to be analyzed using TLC:
    (a) Which is the adsorbent of choice, alumina or silica gel? Explain.
    (b) Considering that the proper adsorbent was used, what would be the best solvent, water or ether, to use? Explain.

ANSWERS

1. (a) The compound will be dissolved away; hence no spots will be seen.
   (b) The spots will show signs of tailing.
   (c) The spots will be expanded due to diffusion.
   (d) Samples that are spotted too close together tend to spread out and run together as they are developed.
2. $R_f = 0.25$
3. (a) 0.55 cm (b) 2.2 cm (Notice how the longer solvent development affords a better separation between spots.)
4. $CH_3(CH_2)_3COOH$, $CH_3(CH_2)_6OH$, $CH_3(CH_2)_{10}CH_3$ (The least polar cmpd. will have the highest R_f because it is more attracted to the nonpolar mobile phase, hexane, than to the polar, silica gel, stationary phase.)
5. The compound will have a very small R_f value (i.e., the compound will not move up the TLC slide very much, if any at all.)
6. To determine: the components in a mixture, the identity of substances, the effectiveness of a purification, the conditions for column chromatographic separation, and the progress of reactions.
7. Very volatile samples (compounds whose bp are roughly less than 130) will evaporate from the plate, and not be able to be detected.
8. (a) Since this compound is not unsaturated, it will neither absorb UV or visible light. Since this compound is an alkyl halide, iodine vapors will not work; therefore, none of the three visualization techniques will work with this compound. However, visualization techniques to detect alkyl halides do exist.
   (b) Since this compound is volatile (bp < 130) it will evaporate from the plate and not be able to be detected at all.
   (c) Since this compound has 9 conjugated bonds it will probably have a color and direct visualization can be used. Since this compound is unsaturated, it will absorb UV light; and since this compound is not an alkane or alkyl halide, it will be stained by iodine vapors.
9. $R_f = 0$ means that the compound did not move off the origin.
10. An $R_f > 1$ is impossible! The student made a terrible mistake.
11. No; because the spots would only be separated by 0.15 cm which is really not that large of a distance. Ideally, what you would like to see is a separation greater than 0.4 cm.
12. (a) Since both compounds are nonpolar alumina is the adsorbent of choice.
    (b) Since both compounds are nonpolar, they would not be expected to dissolve in water, the solvent would not carry these compounds off the origin, and hence the compounds would not be separated. Therefore, the solvent of choice would be ether (which is nonpolar).