Experiment 1 & 2

Separating a Mixture of Anthracene, Benzil and Triphenylmethanol by Thin-Layer Chromatography & Isolation of (-)-Menthol from Peppermint Oil and Its Conversion to (-)-Menthyl Acetate

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PURPOSE OF THE EXPERIMENT

Perform a thin-layer chromatography, a basic technique in organic synthesis including (1) choosing an eluent system, (2) comparing R_f values, (3) identifying compounds.
Use basic column chromatography skill to isolate (-)-menthol. Set up a simple acetylation reaction with the purified menthol and purify (-)-menthyl acetate.

BACKGROUND INFORMATION

Thin-layer chromatography (TLC) is a simple and inexpensive analytical technique that can quickly and efficiently separate less than ten micrograms (μg) of material. In organic laboratory, TLC can be applied in various ways. It can be applied in rapid analysis of reagent and product purity, quick determination of the number of compounds in a mixture and monitoring the reaction progression. Also, by comparing unknown compounds' behavior to the behaviors of known standard compounds, mixture can be tentatively identified.

Chemists frequently use TLC to follow the progress of a reaction by monitoring the disappearance of a reactant or the appearance of a product. Also, TLC often is used to select a suitable solvent before attempting column chromatography. During the column chromatography experiment, TLC is used to monitor the movement of the components.

The term chromatography refers to several related techniques for analyzing, identifying or separating mixtures of compounds. All chromatographic techniques have a two-part operation in common. In each technique a sample mixture is placed into a liquid or gas, called **mobile phase**. The mobile phase carries the sample through a solid support, called **stationary phase** which contains an adsorbent or another liquid. The different compounds in the sample mixture move through the stationary phase at different rates, due to different attractions for the mobile and stationary phases. Thus, individual compounds in the mixture separate as they

move through the stationary phase. The separate compounds can be collected or detected, depending on the particular chromatographic technique involved.

In TLC, capillary action allows a liquid (mobile phase) to ascend a solid (stationary phase) coated on a support **plate**. A sample of the compound mixture is applied near the bottom of a dry TLC plate, as shown in Figure 1(a). The plate is placed into a developing chamber, a covered container with a shallow layer of mobile phase liquid in the bottom. As the mobile phase ascends the plate, the mixture compounds dissolve in the mobile phase to different extents, due to differences in their relative affinities to the mobile and stationary phases. After the separation is complete, the TLC plate is called a chromatogram, as shown in Figure 1(b).

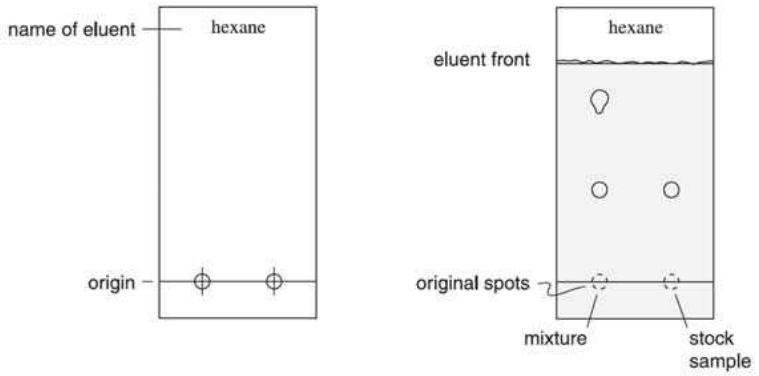


Figure 1. A TLC plate (a) labeled for identification and spotted, and (b) as a chromatogram.

During the TLC process, the solid stationary phase, called the **adsorbent**, adsorbs the mixture compounds. As the mobile phase, called the **eluent**, travels up over the adsorbent the compounds within the mixture move at different rates. A reversible and continuous competitive attraction between the eluent and the adsorbent for the mixture compounds causes this rate difference.

Compounds with less attraction for the adsorbent move rapidly with the eluent. Compounds with more attraction for the adsorbent move slowly with the eluent. Because TLC adsorbents are typically very polar, the more polar is a compound in the mixture, the stronger it adheres to the adsorbent and the slower it moves.

Similarly, intermolecular attractions between the eluent and the compounds determine the solubility of the compounds in the mobile phase. In TLC, the more polar the eluent, the more rapidly a given compound moves. Polar compounds, which are strongly attracted to the adsorbent, require polar eluents to attract them away from the adsorbent.

Determining a Retention Factor

The ratio of the distance that a compound moves to the distance that the eluent front moves is called the **retention factor**, denoted as R_f. A calculation for R_f is shown in Equation 1.

$$R_f = \frac{distance\,traveled\,by\,compound,\,mm}{distance\,traveled\,by\,eluent\,front,\,mm} \qquad \text{(Eq. 1)}$$

For example, in Figure 2 the stock sample compound moved distance A while the eluent front traveled distance S. If distance A is 25 millimeters (mm) and distance S is 55 mm, then the R_f is calculated as shown in Equation 2.

$$R_f = \frac{A}{S} = \frac{25\,mm}{55\,mm} = 0.45 \qquad \text{(Eq. 2)}$$

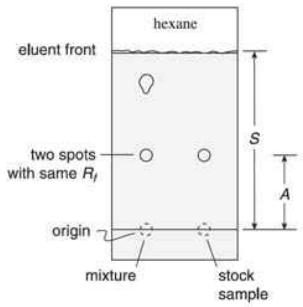


Figure 2. A chromatogram showing measurement for R_f calculations

The chromatographic behavior of individual compounds is reproducible as long as the stationary, mobile phases and temperature are kept constant. Therefore, an R_f can be used for identification purposes.

When a compound is strongly attracted to the adsorbent and does not travel very far from the origin, or point of application, the R_f is small. An increase in eluent polarity would probably increase the attraction of the compound for the eluent. As a result, the compound would move farther up the plate, resulting in a larger R_f.

Identical R_fs for a known compound and an unknown compound on the same chromatogram suggest that the known and unknown compounds are the same. However, two different compounds can have the same R_f in a given eluent. Additional evidence that two samples are the same compound can be obtained by comparing their mobilities in several eluent systems of varying polarities. Two different compounds that have the same R_f in one eluent are unlikely to have the same R_f in other eluents of different polarities, while two different samples of the same compound will have the same R_f in every eluents.

Choosing Adsorbent and Eluents

Alumina (Al_2O_3) and Silica gel ($SiO_2 \cdot xH_2O$) are the most commonly used adsorbents in TLC and column chromatography. However, for use in TLC, a binder such as calcium sulfate is added to these absorbents to hold them onto the plate. For this reason, commercially prepared adsorbents may not be used interchangeably between TLC and column chromatography.

Alumina is generally suitable for chromatography of less polar compounds. Silica gel gives good results with compounds containing polar functional groups.

The eluents are organic compounds of various structures and polarities, as shown in Table 1. The more polar an eluent, the

greater is its **eluting power**, that is, its ability to move compounds over the adsorbent surface.

Combining eluents of low polarity with those of high polarity allows the preparation of mixed eluents of practically an eluting power. For example, the eluting power of a 1:1 mixture of hexane and ethyl acetate would be between the eluting powers of pure hexane and pure ethyl acetate. Eluent selection is usually a matter of trial and error until a separation of desired mobility is achieved.

Table 1. Approximate order of polarity of eluents used in chromatography.

least polar	cyclohexane
	petroleum ether
	hexane
Increasing eluting power	toluene
	dichloromethane
	ethyl acetate
	ethanol
	acetone
most polar	methanol

Using TLC in an Experiment

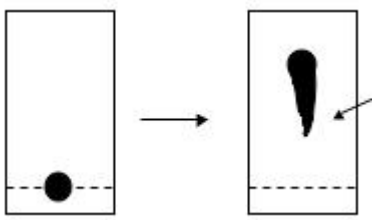
A TLC experiment has three general stages: spotting, developing, and visualizing.

Spotting a Plate

The origin is marked, usually by drawing a thin line across the bottom of the plate with a pencil (Figure 1a). The sample compound or mixture should be dissolved in a volatile solvent such as acetone or dichloromethane. A glass capillary tube is used to apply a small amount of sample solution onto the plate, keeping the sample in as small an area as possible. With practice, spots with diameters of 1-2 mm can be produced.

After the solvent evaporates, additional sample solution can be applied to the same spot. Application of too much sample can lead to "tailing" and poor separation (Figure 3). Varying amounts of a sample can be spotted on the same plate to determine which application gives the best results.

Figure 3. Spotting a plate



Overloading the spot- Location of center inaccurate
Spot too small- Difficult to see
*Check spot under UV light before developing

Developing a Plate

To develop the chromatogram, a piece of filter paper is placed along the walls of the developing chamber, which contains a shallow layer of the appropriate eluent. The paper acts as a which that adsorbs the eluent and ensures that, when the chamber is closed, its atmosphere is saturated with eluent vapor, minimizing evaporation from the plate.

When the spotted plate is placed into the chamber, the origin marked on the plate must be higher than the level of the eluent, to prevent the sample from dissolving from the plate into the eluent layer. When the eluent reaches a point approximately 5 mm below the top of the plate, the plate is removed from the chamber. The point that the eluent has reached is called the eluent front and is immediately marked with a pencil (Figure 1b). The plate is dried by allowing the eluent to evaporate from the plate.

If the eluent front is allowed to reach the top of the plate, the mixture compounds may continue to move along the plate. An R_f measured under these circumstances is not valid.

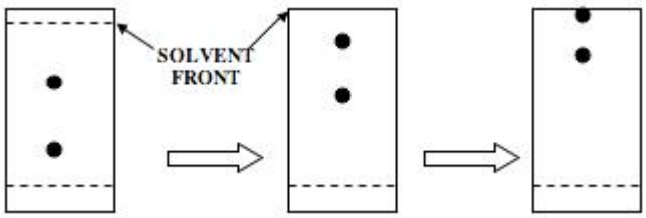


Figure 4. Developing a Plate

Visualizing the Compound

Upon development, a successful separation of colored compounds will reveal distinct spots, indicating that the mixture compounds have separated (Figure 1). To make separated colorless compounds observable to the eye, the sports are treated in some way to make them visible. This process is called **visualization**.

Some compounds fluoresce. Such compounds can be visualized by viewing the TLC plate under an ultraviolet (UV) lamp. Frequently, the adsorbent contains a chemically inert fluorescent material (eg, phenyl groups). When viewed under UV light, compounds that absorb the UV light appear as dark spots that may be outlined with a pencil.

Another simple method for visualizing organic compounds is to place the chromatogram in a chamber containing iodine (I_2) crystals and vapor. The I_2 vapor forms a colored complex with many compounds and allows detection of their spots. The spot location must be marked immediately because the I_2 wil eventually sublime from the plate.

In some instances, a reagent such as potassium permanganate, vanillin, anisaldehyde, or phosphomolybdic acid solution is sprayed on the plate. This reagent formed a colored product with the compound of interest.

Column Chromatography

Like thin layer chromatography, column chromatography employs silica gel as a stationary phase and an organic solvent as a mobile phase. Column chromatography is carried out in a glass tube clamped vertically with the initial mixture placed at the top. Organic moves through this mixture on their way down the column. Because of silica gel's strong affinity with the polar components, the components with the lower polarity will descend ("elute") first through the column. During the chromatography, the polarity of the mobile phase can be slowly increased by varying the solvent mixture. As a result, more polar components will be eluted.

It is important to load as minimum volume of the mixture as possible to avoid overlap of the components when they are eluted. Below the column, test tubes are located to collect fractions. If test tube is filled with eluent, test tube is changed to another clean test tube. Each test tube should be labeled to avoid confusion. Test tubes which contain target product is then collected and evaporated to isolate the product.

Isolation of (-)-Menthol from Peppermint Oil and Its Conversion to (-)-Menthyl Acetate

In this experiment, students will extract (-)-menthol from peppermint oil and synthesize (-)-menthyl acetate by esterification reaction. During the extraction part, they can learn and run flash column chromatography by themselves. In addition, students will use TLC method and understand how to detect the exact partition of (-)-menthol from organic solution.

Peppermint oil *(Mentha* x *piperita L.*), the natural compound, is used as the source of compound (-)-menthol in this experiment. Students can understand that natural plants are the source of medical/chemical compounds by searching application of peppermint oil. Esterification, which is a representative process of chemical alternation, is done by students to understand basic organic synthesis. They can also figure out results of changing conditions (catalyst, solvent, reactant) and practice designing chemical reaction.

Peppermint, which was first recorded in 1696, is a natural hub that is an ingredient of tea and medicine (decongestant, antioxidant, cure headaches, etc). Refreshing scented peppermint oil is extracted from peppermint by steam distillation method. This valuable, friendly peppermint oil will be used in this experiment to be familiar with chemical

experiment methods.
Column chromatography is the basic method used in all of the fields of chemistry. The major course of chemistry should include experiment that makes students familiar with this method. Therefore, in this two-week course experiment will focus on that point. In the first week, students will concentrate on learning flash column chromatography. In the next week, students will utilize column chromatography and extraction methods to perform esterification of (-)-menthol.

EXPERIMENT WEEK 1
Reagents and Properties

substance	*quantity*	*molar mass* (*g/mol*)	*mp* (*°C*)	bp (*°C*)	*density* (*g/mL*)
Anthracene		178.20	218	340	
Benzil		210.23	94	346	1.23
Triphenylmethanol		260.33	160	360	1.199
dichloromethane	~ 3 mL	84.93		40	
ethyl acetate	~ 3 mL	88.11		77	
hexanes	~ 3 mL	86.18		69	
methanol	~ 3 mL	32.04		65	
acetone	~ 3 mL	58.95		56	
peppermint oil	200 uL				0.898
DMAP	78 mg	122.17			
Ac_2O	90 uL	102.09			1.082

PROCEDURE

Caution: Wear lab coats and safety goggles at all times while in the lab. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Wearing contact lens is strictly prohibited.

1. Preparing Capillary Micropipets for Spotting

Caution: Before lighting any flame in the laboratory, check for the presence of any flammable solvents nearby. Extinguish all flames before preparing the developing chambers, which contain flammable eluents.

Note 1. Your TA will demonstrate how to prepare a capillary micropipet.

Prepare micropipets for spotting the TLC plates by drawing out Pasteur pipets. Draw out the Pasteur pipets using a small flame from a Bunsen burner to heat the midpoint of a Pasteur pipet. Slowly rotate the pipet until a yellow flame indicates the pipet is softened. Romove the pipet from the flame and immediately draw out the ends of the pipet to form a very fine open capillary.
Break the capillary using a sand paper.

2. Prepare the Developing Chamber

Caution: Use a ***fume hood***. Prevent eye, skin and clothing contact. Avoid inhaling vapors and ingesting the compounds.

Obtain four 100 mL beakers and label each beaker with the name of one of the eluents: “ethyl acetate”, “hexanes”, “methanol”, and “dichloromethane” Obtain four rubber bands and four pieces of plastic wrap, each large enough to cover a 100 mL beaker.

Using a glass stirring rod to direct the flow, pour ~2-3 mL of the appropriate eluent into each beaker to moisten the filter paper liner and to form a layer of 3-4 mm deep. Cover each developing chamber with a plastic wrap and set aside.

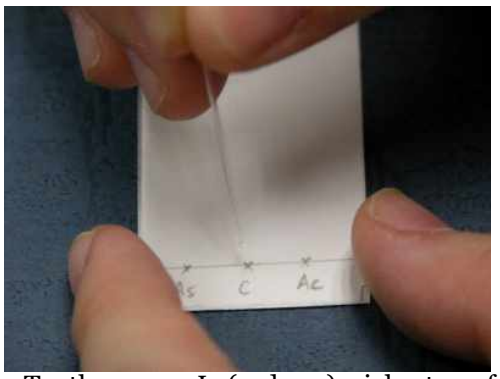
3. Spotting the TLC plates

Obtain 2.0 (3.0) × 4.0 cm TLC silica plates [Note 2]. With a pencil, label each plate at the top with the name of one of the four eluents.

Note 2. Avoid touching the coated surface of the TLC plate with your fingers. Hold the plate by the sides or with a tweezer. Use a pencil to mark on the TLC plate. Inks dissolve in the eluents.

Mark the origin on each of the four plates by marking a very faint pencil line across the plate 0.5-1.0 cm from the bottom. Faintly mark three cross-hatch lines on the origin line to indicate where the solution will be spotted, as shown in Figure 5. Alternatively, you can mark three dots on an imaginary parallel line 0.5-1.0 cm from the bottom. To the three lines or dots, mark A, B and C (or other names to indicate three compounds).

Figure 5.



To three 2 mL (1 dram) vials, transfer 0.5 – 1.0 mL of the stock solution of benzyl, triphenylmethanol and Antracene label the vials. Place the end of a capillary micropipet into the solution and allow the liquid to rise by capillary action. Spot the solution onto one TLC place by quickly and lightly touching the end of the micropipet to the surface of the adsorbent at each cross-hatch or dot. Transfer an amount of liquid to the plate that produces a pot with a diameter less than 2 mm. Allow the solvent to dry completely between applications to the same spot. Otherwise, the spot will become too large.

Note 3. You may use new capillary micropipets for new compounds, but you can safely clean the micropipet using acetone.

After loading one compound, rinse the capillary micropipet with acetone at least two times [Note 3]. Then, repeat spotting of other two compounds in the same TLC plate.

4. Developing TLC plates

Caution: Ultraviolet radiation can cause severe damage to the eyes. Wear goggles. Do not look directly into the UV lamp.

Check to be certain that the eluent level in each developing chamber is *below* the point where the samples have been spotted. Place the spotted TLC plate into the ethyl acetate developing chamber. Use the chamber wall to support the plate, as shown in Figure 6. Cover the chamber with a piece of plastic wrap, secured with a rubber band.

When the eluent rises to within 5 mm of the top of the plate, remove the plate from the chamber and *immediately* mark the eluent front with a pencil. Allow the eluent to evaporate from the plate under a fume hood.

Figure 6.

Examine the developed TLC plate under a UV light (254 nm). Use a pencil to circle any visualized spots. A reagent such as anisaldehyde or phosphomolybdic acid solution is sprayed on the plate. This reagent formed a colored product with the compound of interest.

Use a ruler to measure the distance from the origin to the center of each spot on the plate developed with the chosen eluent. Measure the distance from the origin to the eluent front. Use Equation 1 to calculate the R_f values for each spot.

Repeat this process for other three solvents and fill out the table below.

5. Identification of the eluent system for the separation of anthracene, benzil, and triphenylmethanol.

***R_f* values**

	Hex	EA	DCM	MeOH
Anthracene Benzil Triphenylmethanol				

Hex: hexanes, EA: ethyl acetate, DCM: dichloromethane
MeOH: methanol

What is the best eluent solvent to separate three compounds

at the same TLC plate? If there is no answer, try solvent mixtures of hexanes and ethyl acetate (25:1 to 10:1).

6. Identification of components in the mixture solution.

You will be given 2 different solutions (solution A and solution B) containing anthracene, benzil, triphenylmethanol, or any combination of these chemicals. Identify the chemical(s) present in each solution.

Solution A:
Solution B:

7. Preparing the Column (Wet-pack Method)

Using a utility clamp, attach the column to a support stand. Use a glass rod to push the cotton at the bottom of the column. Be careful not to make the plug too tight or the eluent flow will be limited. Make sure the column is vertical. If the clamp is too large to firmly hold the column, use paper towels or a split stopper to hold the column in the clamp tightly. Add a small amount of sea sand on the cotton.
Slowly add hexane to the beaker containing silica gel and mix until the mixture become slurry. Make sure that stopcock is closed, and then pour the slurry of silica gel into the column using funnel. The height of the packed silica gel in column allows to be ~13 cm high. Open the stopcock and wash inside of the column with additional hexane. Allow the solvent to be drained through the column until its level is slightly above the the silica gel. (***CAUTION*** : No cracks and bubbles should be formed.) Then, pour a little amount of sea sand on the silica gel to make thin layer (<5mm) of sea sand above the silica gel.

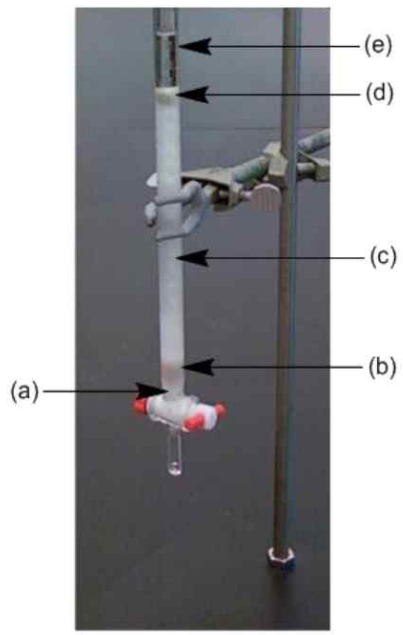


Figure 3.1. Prepared chromatography column

8. Choosing the eluent using TLC experiment

a: cotton, b: sand, c: silica gel, d: sand. e: mobile phase
According to the experiment TLC, develop the TLC. Choose the appropriate solvent system based on the result from TLC experiment.

Note. It is possible to change the polarity of the solvent system during separation, by changing the ratio of the solvent. Generally, the polarity of the solvents should be changed gradually from a less-polar to a more-polar solvent. (So-called gradient column chromatography)

9. Load the Sample to the Column

Load peppermint oil (0.2 mL). Make sure that the surface of the column is flat while loading the sample. Then add small amount of sea sand above the loaded sample.

10. Running the Column

Caution: Once the column chromatography has started, it should not be stopped or allowed to run dry for any reason! You should monitor the column and collect fractions, and prepare the necessary eluting solvent well in advance of its use.

Label 20 ml vial as 1,2,3 and 10. These vials will be used to collect the components (fractions) of the mixtures as they are eluted from the column.

Carefully transfer the appropriate eluent, consists of ethyl acetate and hexane, into the column. Make sure not to disturb the silica layer when adding the eluent.

Collect the initial colorless eluent in beaker (~50 mL). This is fraction #1. Collect in another vial as fraction #2.
Continuously collect fraction and re-fill the eluent until all of the compounds are come out from the column. It can be monitored by TLC.
If you separate one compound from the mixture, you can use more polar solvent mixture as an eluent.

11. TLC

The mixture can also be analyzed with U.V-VIS spectroscopy.

Develop all the fraction that you collected on a silica gel TLC plate with standard solution.

Calculate and report the R_f values for all spots on all plates. You have to note eluent ratio of each fractions you collected, and should check which fractions are pure. Discuss about it in results and discussion.

Submit your TLC plates according to the directions on the Report Form.

12. Isolation of (-)-Menthol

Transfer the fractions which contain (-)-menthol into a pre-weighed round bottomed flask(RBF). It is recommended that the volume of the solution is not more than half volume of the RBF. Rinse the vial with small amounts (2 x 2 mL) of EA.

Evaporate the solvent using the RotoVap. Weigh the RBF containing (-)-menthol. Calculate the weight of (-)-menthol recovered from column chromatography.

13. Characterizing the Product

Using NMR Spectroscopy

Caution: Deutero-Chloroform is toxic and a suspected carcinogen. Dispense it in a fume hood. Wear protective gloves.

Product was dissolved in deutro-chloroform. Obtain a NMR spectrum of your product as directed by your laboratory instructor.

14. BEFORE YOU LEAVE THE LAB

Turn off any electrical equipment that you may have used, put away your equipment and lock your drawer, clean up your work areas, close the fume hood sash completely, and ask your TA for her or his signature. In general, please try to keep the lab in as good condition as you found it. If you see spilled chemicals, clean them up or at least report it to your TA.

Waste

Never dispose of glass waste in the regular trash. The custodian could become injured. Dispose of all glass waste in the cardboard "Glass Only" boxes. Used capillary pipets may be placed into those boxes also or into the dishes placed around the lab for that purpose. Dispose of all solutions and developing solvents in the ORGANIC LIQUID WASTE container in the hood. When finished, pour as much of the solvent as possible into the waste container and leave the jars open in the hood so they will dry.

EXPERIMENT WEEK 2

1. Acetylation of (-)-Menthol

Put DCM, menthol, DMAP and Ac_2O in the 10ml R.B and start the reaction at r.t. Check the progress with TLC. After the reaction is done (about 1hr), work up with H_2O.

Note.

Instructor may prepare commercial (-)-menthyl acetate for the TLC spot.

DCM was added, and use separatory funnel to extract dichloromethane layer.

Column chromatography was done with eluent (Hex : EA = 20:1).

Note.

There are some other conditions which also exist in the original paper. Instructor may alter the condition if needed.

2. Characterizing the Product

Using NMR Spectroscopy

Caution: Deutero-Chloroform is toxic and a suspected carcinogen. Dispense it in a fume hood. Wear protective gloves.

Product was dissolved in deutro-chloroform. Obtain a NMR spectrum of your product as directed by your laboratory instructor.

3. Cleaning up

Clean glass wears and all the instruments. Instructor should check each student's hood.

Post-Laboratory Questions

Experiment 1

1. Explain why the compounds have different R_f values in TLC plate.

2. Explain each.
(a) Why (-)-menthol is not active at UV lamp (254 nm) different from benzil, triphenylmethanol and anthracene? (descirbe in detail) (b) If then, how to visualize the (-)-menthol peak at TLC?

3. Circle the right answer.
For developing the TLC, utilization of polar solvents (increase/decrease) R_f values. And nonpolar compounds usually have (high/low) R_f values.

4. Explain the reason why a minimum amounts of solvent should be used when you load a sample to the column.

5. Before starting the column chromatography, you have to choose a column with proper height and proper width. What are the disadvantages of short and narrow column?

Experiment 2

1. Draw the reaction mechanism of the acetylation reaction

(describe the role of DMAP).

2. Explain the reason why we should do work up process in this experiment.

3. Analyze NMR spectra of (-)-Menthol and (-)-Menthyl acetate. Mark the characteristic peaks of (-)-Menthyl acetate from (-)-Menthol.

Pre-Laboratory Question Experiment 1 (1st week of experiment)

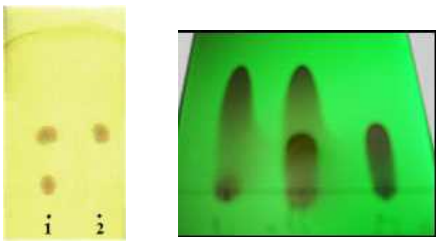
Only handwriting! No typing.

1. Summarize MSDS of all chemicals used in this experiment.

2. Draw chemical structures (TLC staining solutions) of anisaldehyde, ninhydrin and phosphomolybdic acid, potassium permanganate solution. Which functional groups are active for each reagents in principle?

3. List the order of polarity of eluents used in chromatography (cyclohexane, hexane, toluene, dichloromethane, ethyl acetate, ethanol, acetone, methanol, water).

4. Generally, the shape of TLC spot seems like a dot but sometimes it gave draggy spot called 'tailing'. Why 'tailing' takes place and how to avoid or minimize it?



Normal TLC spots (left) and tailing spots (right).

Pre-Lab Question Experiment 2 (2nd week of experiment)

1. Describe each

(a) Explain the reactivity difference of acetyl chloride and acetic anhydride. Which is more reactive?

(b) Describe the general reactivity order of aldehyde, acid, amide, acid chloride and anhydride.

2. Draw the structure of DMAP and explain the more nucleophilic position (between two nitrogen).