Thin Layer Chromatography

Introduction

Chromatography is an effective and very useful method for separation and purification of organic compounds that can be used even for complex mixtures. Chromatography separates components of a mixture based upon how well they are adsorbed on an inert phase (the stationary phase) versus how well they dissolve in a liquid phase (the mobile phase) (Figure 1). A mixture is introduced to a chromatography system and the components with greater affinity for the mobile phase (white circles) will move (elute) faster than those components with greater affinity for the stationary phase (black circles), causing the components to separate.

![Diagram of chromatography](image)

Figure 1. An illustration of chromatographic separation: Black circles have greater affinity for the stationary phase. (a) - (d) Different affinity for the stationary phase versus affinity for the mobile phase ultimately leads to separation.

The stationary phase is a solid, inert material that contains a polar functional group, and therefore polar compounds have a greater affinity for the stationary phase. Because the selection of stationary phases is rather limited, it is the mobile phase (an organic solvent) that usually is changed, which is easy to do. The best solvent depends on the mixture of compounds to be separated, and may range in polarity from alkanes (for example, hexane) to alcohols (methanol). An increase in the polarity of the mobile phase increases the rate of elution for all compounds in a sample. Therefore, if the compounds elute too slowly, the remedy is to increase the polarity of the mobile phase. On the other hand, if the mixture doesn't separate well because all components move too fast, a switch to a less polar solvent is needed. Often, just the right polarity is achieved by mixing a small amount of a polar solvent with a non-polar one. The optimum mobile phase mixture is determined by trial and error, usually using thin-layer chromatography, as discussed below. Your lab class will determine which mixture of dichloromethane (more polar) and pet ether (less polar) provides the best separation of tomato pigments.

In this laboratory, you will experiment with thin layer chromatography (TLC), in which the stationary phase is coated on a plate of glass or plastic.

Thin Layer Chromatography (TLC)

Figure 2 illustrates thin layer chromatography (TLC), the simplest and fastest liquid chromatography technique. A spot of the mixture to be separated is placed on the baseline near the bottom of the TLC plate. The liquid mobile phase (either a single solvent or mixture of solvents) is drawn up the plate, passing the spot of the mixture, when the TLC plate is placed in a developing chamber, which contains the mobile phase. The solvent will be drawn up through the stationary phase by capillary action, and the solvent front moves up the plate. As the solvent passes the spot where the sample was applied, the sample will begin partitioning between stationary and mobile phases, and separation will occur (see Figure 2). The ratio of the distance traveled by a particular spot, compared to the distance traveled by the solvent front (both measured from the baseline) is called the RF value. The best separation of components generally is achieved when the solvent polarity is adjusted so that the compounds being separated have RF values in the range 0.4 - 0.6. RF values are helpful in identifying compounds, especially when you have an authentic sample for comparison.

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The developing chamber can be as simple as a covered beaker (see Figure 3). Keep the depth of the solvent below low the level of the baseline. To maintain the developing chamber saturated with solvent vapor, place a piece of filter paper along the inner wall of the beaker - this serves as a wick to ensure solvent saturation. Set the plate in the chamber on the baseline edge.

It is very important to take care during sample application to keep the sample spot small. You will observe clear separation when the spot applied at the baseline is small (Figure 2c), but if the original spot is too large (Figure 2d), the components will be run together.

You will use TLC later in the semester to monitor the progress of a reaction - as the reaction proceeds, the spot due to the starting material diminishes, and that of the product dominates. Side products or impurities are evident as additional spots. In this experiment, you will separate colored pigments, and most of the spots will be visible to the eye.

However, many organic compounds are colorless, and the spots on the TLC plate must be visualized differently. Some compounds fluoresce and are visualized under an ultraviolet (UV) lamp. A different technique involves the use of TLC plates that have a fluorescent material bound to the stationary phase. Many organic materials prevent (quench) the fluorescence. When observed under an ultraviolet lamp, the plate glows, except for dark spots that mark the location of the organic compounds. Another method is to place the developed plate in ajar containing a few crystals of iodine. Iodine vapor stains the organic compounds brown.

A key limitation of TLC is that the amount of material that can be separated is quite small, so TLC is normally used for visual analysis of mixtures, but not to obtain pure samples. Preparative-scale purifications are more typically accomplished by column chromatography.

Figure 3. A Beaker as a TLC developing chamber. The filter paper helps keep the air saturated with solvent, so the plate will not dry out.
Safety Notes!

Ethanol and petroleum ether are extremely flammable! Keep them away from flames and sources of electrical spark.

Dichloromethane is toxic and has been categorized as a carcinogen. Avoid inhalation, ingestion, and contact with skin. In case of accidental contact, wash with copious amounts of water. All solvents and other waste materials must be discarded in the containers provided.

A. Extracting Pigments from Tomato Paste

Weigh 6 g of tomato paste and 4 g of strained carrots into a small Erlenmeyer flask. Record the weight. Add 10 mL of 95 % ethanol (The alcohol will carry away most of the water.), and mix thoroughly with a spatula. Remove the dehydrated pastes with a spatula, squeezing out as much of the ethanol as possible, and place the resulting paste on a filter paper. Fold the filter paper and squeeze the paste further. Transfer the paste into a dry Erlenmeyer flask. Add 10 mL of dichloromethane and again mix thoroughly with a spatula. Allow the pastes to sit in the dichloromethane, with occasional stirring, for 5 more minutes. Transfer the dichloromethane containing the pigments to a clean Erlenmeyer flask using a disposable Pasteur pipette. Add 1 g of anhydrous sodium sulfate to the dichloromethane mixture and swirl the flask - add more if necessary to obtain a clear, dry solution. Filter the dry organic solution through a funnel fitted with a very small cotton plug, collecting the solution in a 25 mL round bottom flask. Remove most of the solvent using a rotary evaporator under vacuum, leaving approximately 0.5- 1 mL of solvent. Add 1 mL of petroleum ether to the concentrated solution for TLC analysis.

B. TLC Analysis of Pigments in Tomatoes and Carrots

Keep all solvent mixtures covered to minimize evaporation, which would cause a change in the composition of the mixture. You will run ALL 3 solvent systems to note the difference in the resulting Rf.

- 5% dichloromethane/95% pet ether
- 10% dichloromethane/90 % pet ether
- 15% dichloromethane/85 % pet ether

Curl a small (7 cm) circle of filter paper around the inside wall of a clean, dry 150 mL beaker (see Figure 3). Prepare 10 mL of the solvent system assigned to you, and add 8 mL (a depth of about 3 mm) to the beaker. Swirl to facilitate saturation of the chamber with solvent vapor, cover with a watch glass, and let stand for a few minutes. Obtain a silica gel thin-layer chromatography plate. Draw a pencil line across the width, 7 mm from the bottom edge. Dip a TLC spotting capillary into the pigment concentrate (Be certain that pigment solution has been drawn into the capillary!), briefly touch the end of the capillary to one of the TLC plates on the pencil line, and blow gently on the resulting spot to evaporate the solvent. Make two additional spots, spaced about evenly apart across the bottom of the plate on your baseline. Use 3 and 9 touches, respectively, to achieve substantially different amounts of sample on the three spots. (The heavier applications might reveal more minor components.) Stand the plate with the baseline edge down in the developing chamber - The spots must be above the solvent level - replace the watch glass, and watch the solvent front rise up the plate. When the solvent front has reached within 1-2 cm of the top of the plate, remove the plate, and mark the position of the solvent front. (It will dry quickly.) Circle the visible spots with a pencil - they tend to fade. You should be able to see the red-orange lycopene, perhaps the yellow b-carotene, and perhaps others. Develop the plate in an iodine chamber and mark any additional spots that show up. (Check the baseline, too.) Determine the Rf values for all the spots, make an accurate sketch of the TLC plate in your notebook, and record the Rf values. Compare the Rf differences with your lab mates that used a different solvent system.
PART 2 of the Chromatography Experiment

PLEASE ADD ALL OF THESE REAGENTS IN PART 2 TO THE REAGENTS TABLE: THERE ARE AT LEAST 5!!

Objective

Esters are the product of reaction of an organic (carboxylic acid) with an alcohol. Many esters are components of the essential oils of flowers and fruits. Several esters with pleasant fragrances will be synthesized in this experiment, and a common fragrant ester will be hydrolyzed to demonstrate the reverse of the esterification reaction. You will monitor a chemical reaction using TLC to see how the materials present change over time. **NOTE: you will NOT have to do the mechanism for esterification reactions.**

Introduction

When an organic acid, R-COOH, is heated with an alcohol, R’-OH, in the presence of a strong mineral acid, the chief organic product is a member of the family of organic compounds known as esters.

The general reaction for the esterification of an organic acid with an alcohol is

\[ R-COOH + HO-R' \rightarrow R-CO-OR' + H_2O \]

In this general reaction, R and R’ represent hydrocarbon chains, which may be the same or different. As a specific example, suppose acetic acid, CH₃COOH, is heated with ethyl alcohol, CH₃CH₂OH, in the presence of a mineral acid catalyst. The esterification reaction will be

\[ CH_3-COOH + HO-CH₂CH₃ \rightarrow CH₃-COO-CH₂CH₃ + H₂O \]

The ester product of this reaction (CH₃-COO-CH₂CH₃) is named ethyl acetate, indicating the acid and alcohol from which it is prepared. Esterification is an equilibrium reaction, which means that the reaction does not go to completion on its own. Frequently, however, the esters produced are extremely volatile and can be removed from the system by
distillation. If the ester is not very easily distilled, it may be possible instead to add a desiccant to the equilibrium system, thereby removing water from the system and forcing the equilibrium to the right.

Unlike many organic chemical compounds, esters often have very pleasant, fruitlike odors. Many of the odors and flavorings of fruits and flowers are due to the presence of esters in the essential oils of these materials. The table that follows lists some esters with pleasant fragrances, and indicates from what alcohol and which acid the ester may be prepared.

The esterification reactions shown above are actually equilibrium processes and can be reversed. The reverse of the esterification reaction is referred to as a hydrolysis reaction, because it represents the breakdown of the organic compound through the action of water.

\[
\text{R-CO-OR'} + \text{H}_2\text{O} \rightarrow \text{R-COOH} + \text{HO-R'}
\]

**Table of Common Esters**

<table>
<thead>
<tr>
<th>Ester</th>
<th>Aroma</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-propyl acetate</td>
<td>pears</td>
<td>n-propyl alcohol/acetic acid</td>
</tr>
<tr>
<td>methyl butyrate</td>
<td>apples</td>
<td>methyl alcohol/butyric acid</td>
</tr>
<tr>
<td>octyl acetate</td>
<td>oranges</td>
<td>n-octyl alcohol/acetic acid</td>
</tr>
<tr>
<td>ethyl butyrate</td>
<td>pineapples</td>
<td>ethyl alcohol/butyric acid</td>
</tr>
<tr>
<td>benzyl acetate</td>
<td>peaches</td>
<td>benzyl alcohol/acetic acid</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>sweet-balsamic odor</td>
<td>benzyl alcohol/benzoic acid</td>
</tr>
</tbody>
</table>

Generally a fruit or flower may contain only a tiny amount of ester, giving a very subtle odor. Usually, the ester is part of some complex mixture of substances, which, taken as a whole, have the aroma attributed to the material. When prepared in the laboratory in relatively large amounts, the ester may seem to have a pronounced "chemical odor, and it may be difficult to recognize the fruit or flower that has this aroma.

**Safety Precautions**

- Most of the organic compounds used or produced in this experiment are highly flammable. All heating will be done using a hotplate, and no flames will be permitted in the laboratory.
- Sulfuric acid is used as the catalyst for the esterification reactions. Sulfuric acid is dangerous and can burn skin, eyes, and clothing very badly. If it is spilled, wash immediately before the acid has a chance to cause a burn, and inform the instructor.
- The vapors of the esters produced in this experiment may be harmful. When determining the odors of the esters produced in the experiment, do not deeply inhale the vapors. Merely waft a small amount of vapor from the ester toward your nose.
Apparatus/Reagents Required

Hotplate; 50% sulfuric acid for the preparation of fruit and flower aromas, short-stem disposable plastic pipets,

Procedure

- Set up a water bath in a 250-mL beaker on a hotplate in the exhaust hood. Most of the reactants and products in this choice are highly flammable, and no flames are permitted in the lab during this experiment. Adjust heating control to maintain a temperature of around 70°C in the water bath.

- Some common esters, and the acids/alcohols from which they are synthesized, were indicated in the table in the Introduction. You will synthesize the ester benzyl benzoate and note the aroma of the ester.

- To synthesize this ester, mix 5-6 drops (or approximately 0.2 g if the add is a solid) of the benzoic acid with 15 - 20 drops of benzyl alcohol in a clean, dry test tube.

- Add 1 drop of 50% sulfuric acid to the mixture.

- You will run tlc plates of this experimental mixture at 5 minutes, 15 minutes, and 30 minutes. The solvent system to develop the plates will be 3:1 hexane: ethyl acetate. You will need to observe the tlc under UV light and draw sketches of the tlc plates in your notebook. You will calculate the Rf of each component and the resulting ester. Use the iodine chambers as well.

POSTLAB: In your discussion, you want to communicate how liquid chromatography really works and that you could change the Rf of the components in the mixtures without changing the stationary (polar phase: silica, in our cases) phase.