Thin Layer Chromatographic Analyses

In this laboratory exercise we will analyze a commercial pain reliever to determine the identity of the active analgesic in the reliever’s prescription. We will limit ourselves to non-narcotic analgesics that contain Ibuprofen, Acetaminophen, Aspirin or Caffeine. We will separate the active ingredient(s) using Thin Layer Chromatography and compare the results with standards run on the same chromatogram.

As we have discussed before, Chromatography is the general name applied to a series of separation methods that employ a system with two phases of matter; a mobile phase and a stationary phase.

Analytes in a mixture to be separated interact with the stationary phase with different affinities. While moving through the system, carried along by the mobile phase, those analytes with a low affinity for the stationary phase will tend to move along rapidly, while those with a high affinity will tend to lag behind. Thus, the separation of analytes in chromatographic systems is based on the differential affinity of the analyte for the stationary vs. mobile phases.

Thin Layer Chromatography (TLC) is a fast and inexpensive form of chromatography that has many uses in the organic laboratory. Amongst these are to:

- Identify the Components in a Mixture
- Monitor a Chemical Reaction
- Identify the Proper Conditions for a Column Chromatographic Separation
- Purify a Sample as part of a Preparative Procedure

The stationary phase in TLC is typically an adsorbant made of Silica Gel or Alumina layered onto a glass, plastic or aluminum foil plate. The sample to be analyzed is spotted onto the plate and then placed in a beaker or other chamber with eluting fluid. The eluting fluid, or mobile phase, rises up the plate via capillary action, carrying the analytes with it.
As mentioned, the two most common coatings for thin-layer chromatography plates are Alumina (Al₂O₃) and Silica Gel (SiO₂). These are the same adsorbents most commonly used in Column Chromatography for the purification of macroscopic quantities of material. (In fact, TLC can be thought of as a micro version of column chromatography.) Of the two, Alumina, when anhydrous, is the more active, that is, it will adsorb substances more strongly. It is the adsorbent of choice when the separation involves relatively nonpolar analytes such as hydrocarbons, alkyl halides, ethers, aldehydes, and ketones. To separate the more polar substrates such as alcohols, carboxylic acids, and amines, the less active adsorbent, Silica Gel, is used. In extreme situations, very polar substances on Aluminum Oxide do not migrate very far from the starting point and nonpolar compounds travel with the solvent front if chromatographed on Silica Gel. These extremes of behavior are markedly affected, however, by the solvents used to carry out the chromatographic separation. A polar solvent will carry along with it polar analytes and nonpolar solvents will do the same with analytes that are themselves nonpolar.

Common solvents used in chromatography, both thin-layer and column, are listed below. The higher a solvent’s Dielectric Constant the more polar it is.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Notation</th>
<th>Dielectric Const</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>H</td>
<td>1.9</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>PE</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>Cy</td>
<td>2.0</td>
</tr>
<tr>
<td>Carbon Tetrachloride</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>Benzene</td>
<td>B</td>
<td>2.3</td>
</tr>
<tr>
<td>Toluene</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>DE</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Chloroform  |  Ch   |  4.8
Ethyl Acetate |  EA   |  6.0
Acetic Acid   |  AA   |  6.2
Isopropyl Alcohol |  |  18.3
Acetone       |       |  20.7
Ethanol       |       |  24.3
Methanol      |  M    |  32.6
Water         |       |  78.5

In general, these solvents have low boiling points and low viscosities, so they migrate rapidly along a TLC plate. They are listed in order of increasing polarity. Mixtures of these solvents are often used to “fine-tune” the separation of analytes. Some suggested mixtures for specific classes of organic compounds to be separated on Silica Gel are:

<table>
<thead>
<tr>
<th>Compound Class</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>Cy:EA (1:1 or 1:2) or P:De (10:1)</td>
</tr>
<tr>
<td>Amides</td>
<td>EA:M (5:1)</td>
</tr>
<tr>
<td>Amines, Amino Acids</td>
<td>M:Ch (2:3) + 1% (vol) of 33% Ammonia</td>
</tr>
<tr>
<td>Carboxylic Acids</td>
<td>Ch + 90% Formic Acid to saturation</td>
</tr>
<tr>
<td>Esters</td>
<td>Cy:EA (1:1 or 1:2)</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>P or Cy or B:DE (2:1) or Cy:EA (3:1)</td>
</tr>
<tr>
<td>Ketones</td>
<td>Cy:EA (1:1 or 1:2) or DE:H (1:9)</td>
</tr>
</tbody>
</table>

(Adapted from *Theory and Practice in the Organic Laboratory* by John A. Landgrebe)

Similar solvent mixture suggestions are available for TLC plates made of Alumina.

Once the chromatogram has been run, visualization of the compounds on the TLC plate becomes a problem. In some cases, like the dyes in Ink, the compounds are naturally colored and no visualization procedure needs to be performed.

In other cases, a specific coloring reagent can be applied to the plate. This coloring reagent will react with the analytes to produce visible spots on the plate. In the example below, Vanillin is used to make visible the compounds in the separated Essential Oils.


In more general cases, a fluorescent compound, such as Manganese activated Zinc Silicate, is added to the adsorbant. When placed under a UV lamp, the adsorbant will fluoresce a green color. Analytes will tend to quench this fluorescence, producing a dark spot where analyte is present. (In some cases the fluorescence is enhanced, producing a bright spot on the plate.) Or, the TLC plate can be developed in a chamber containing a few crystals of Iodine that is heated. The Iodine vapor can react with the analyte, producing a brown spot on the plate.

Finally, a chromatogram is typically characterized by the Retention Factors of each analyte and its ability to Resolve the analytes. The Retention Factor ($R_f$) is simply the fractional distance the analyte spot moves along the plate relative to the Solvent Front.
Resolution is a measure of how well the analyte spots separate from each other. Certainly, for good resolution, the analytes should have distinct $R_f$ values. However, the Breadth of the spot also affects the chromatogram’s resolution. As each compound migrates, its spot broadens as material diffuses away from its center. The reasons for this broadening are complex and will not be discussed here. Note only that the longer the system is allowed to develop, the broader will be the spots. Good resolution requires that each spot have minimal breadth. Selecting a chromatographic system, adsorbant and eluent, such that all the analytes have distinct $R_f$’s and minimal spot broadening can be tricky. It is as much art as science.

In this lab we will be separating and identifying the active ingredients in an Analgesic. Analgesics are substances that relieve pain. The most common of these is Aspirin, a derivative of Salicylic Acid. Other common analgesics include Acetaminophen and Ibuprofen as well as a number of narcotics. In many cases these analgesics are used in combination to enhance or complement their individual affects; e.g., Codeine and Acetaminophen. Additionally, to counteract the acidic properties of Aspirin, an inorganic buffering agent is added to some preparations. In some cases, Caffeine is added to counteract the sedative effects of the analgesic. Finally, each commercially prepared analgesic tablet usually contains a binder, often starch, microcrystalline cellulose, or silica gel.
Thus, we will prepare a commercial analgesic tablet for analysis via TLC. The above mentioned compounds will also be run on the same TLC for comparison. The analyte spots will then be visualized under UV light. The $R_f$ value for each standard and each analyte spot produced by the commercial analgesic tablet will be determined. The active ingredient(s) of the commercial tablet will then be identified.
Pre-Lab Questions

1. Suppose you carry-out a TLC separation of Acetaminophen and Phenacetin on Silica Gel using a non-polar eluting solvent. Which should have the larger $R_f$ value and why?

2. A TLC plate showed two spots A and B with $R_f$ values of 0.20 and 0.30, respectively. Sketch a TLC chromatogram with the above $R_f$ values. Assume the TLC plate is 10 cm long, the spots start 1 cm from the bottom of the plate and the solvent runs to within 1 cm of the top of the plate. Be as quantitative as possible.

3. What are the largest and smallest $R_f$ values possible? What physical situation will lead to each?

4. Besides a direct comparison of the unknown with the standards, there is a second reason the standards are run on the same TLC plate as the unknown. What is this reason?
Procedure

1. Prepare your chromatography tanks. Obtain two chromatography Developing Jars.

   **In the fume hood**, add eluting solvent to a depth of about 5 mm in the chamber. We will use two different solvent systems to develop our chromatograms: H:EA (1:1) and EA:AA (95:5). Cover the chamber with its lid and slosh the solvent up the sides of the chamber to saturate the vapor inside the chamber with solvent. Set the chamber aside while you prepare the chromatography plate.

2. Prepare the TLC plate. We will use Whatman Polyester backed plates coated with Silica Gel to a thickness of 250 µm. These plates have been activated to fluoresce under UV\textsubscript{254} radiation. Handle the plates only by the edges. With a paper cutter, cut two TLC plates to 55mm x 90mm. Using a pencil, very, very, lightly draw a line about 1 cm from the bottom edge of the plate. Mark off four 1 cm intervals, starting ~5mm from each edge of the plate. Lightly label each: Ace, Asp, Caff, Ibu, Unk.

   Your instructor will demonstrate how to draw out a melting point capillary tube into a micropipet. Using a separate micropipet, spot your unknown analgesic and each standard analgesic onto the TLC plate. Do this by touching the tip of the micropipet filled with sample to the plate’s surface four or five times. It is important the spot be reasonably concentrated and small. Do not allow the tip of the micropipet to touch the surface for more than a fraction of a second. If it does, it will produce a spot that is too large. And, allow the spot to dry before spotting it again.

   Each standard solution has been prepared by dissolving the standard in a 1:1 Ethanol:Dichloromethane mixture.

   To prepare the unknown analgesic, obtain roughly one-half an analgesic tablet. Prepare a 9 cm pipette with a small cotton plug for filtration. Crush the sample using a pestle in a mortar. Collect the powder in a small beaker or Erlenmeyer flask. Dissolve in 5 mL of 1:1 ethanol/dichloromethane. Pass the sample thru the pipette containing the cotton plug. This will separate any insoluble material from the material soluble in the ethanol/dichloromethane mixture. Collect in a medium test tube. Rinse the beaker or Erlenmeyer flask with another 5 mL of 1:1 ethanol/dichloromethane and pass the solution thru the pipette containing the cotton plug.

3. Develop the chromatogram. Add the TLC plate to the developing chamber. Allow it to run until the solvent front is a reasonable distance (~1 cm) from the top of the plate.
Remove the plate from the TLC chamber and allow it to dry. Be sure to mark the position of the solvent front.

4. Observe the plate under a UV lamp. **Do not look directly into the lamp as UV radiation can cause blindness.** Lightly circle each spot with a pencil.

5. Accurately sketch the thin-layer chromatograms in your notebook. Calculate the \( R_f \) value for each spot. Decide which analgesic(s) and/or caffeine is (are) in your unknown sample.
Post Lab Questions

1. Why is it necessary to run a TLC experiment in a closed container and to have the interior vapor saturated with the solvent?

2. Why must the spot applied to a TLC plate be above the level of the developing solvent? What problem will ensue if the level of the developing liquid is higher than the applied spot in a TLC analysis?

3. What will be the result of applying too much compound to a TLC plate? Not enough?

4. What will be the appearance of a TLC plate if a solvent of too low of a polarity is used for its development? Too high of a polarity?

5. Why is it necessary to add a bit of Acetic Acid to our Ethyl Acetate eluting fluid; recall, we used a 95:5 (EA:AA) mixture?