Experiment 1: Thin Layer Chromatography

Part A: understanding R<sub>f</sub> values
Part B: R<sub>f</sub> values & solvent polarity
Part C: R<sub>f</sub> values & compound functionality
Part D: identification of commercial food dye components

Reading:  MHS  Ch. 17 pgs 219-235
Read Also: MHS  Ch. 1, pp. 3-13
            Ch. 2, pp. 20-21
            Ch. 4, pp. 34-38
            Ch. 5, pp. 38-47
Chromatography

"color writing"

• A variety of techniques used for the separation, isolation, & identification of the components of a mixture

• First described in 1903 (M.S. Tswett) as a method for the separation of plant pigments

• The fundamental basis for chromatography concerns the distribution of the individual components of a mixture between two phases:

  1. stationary phase
     - a non-moving substance to which the components of a mixture adsorb
     - commonly SiO$_2$ or Al$_2$O$_3$

  2. mobile phase
     - gas or liquid
     - percolates over the stationary phase carrying components along in the direction of flow
Chromatography

• So: components adsorbed on the stationary phase do not move components dissolved in the mobile phase move with flow

• Separation occurs because each component of a mixture has a different affinity for the stationary phase, and thus will be adsorbed to a greater or lesser extent than the other components
  - adsorption depends on interaction of specific component with stationary phase
  - stronger interaction = more molecules adsorbed on stationary phase, less in mobile phase

• Effectively establish an equilibrium for each component:

\[ \text{A}_{\text{(mobile)}} \xleftrightarrow{} \text{A}_{\text{(stationary)}} \]

• Differences in equilibrium allow separation
Chromatographic Separation

- Consider a 2-component mixture (A + B):
  - more B in mobile phase
  - ∴ B moves faster than A
  - establish equilibrium
  - adsorption A >> B
  - more B in mobile phase
  - ... B moves faster than A
  - component separation increases with distance mobile phase travels
Types of Chromatography

1. Thin Layer Chromatography (TLC)
   - stationary phase: spread over glass or plastic sheet
   - mobile phase: liquid; drawn up plate by capillary action

2. Column Chromatography
   - stationary phase: contained in a column
   - mobile phase: liquid; passes through column (gravity or pressure)

3. Gas Chromatography (GC)
   - stationary phase: contained in a column
   - mobile phase: gas; passes through column (pressure)
Factors that Affect TLC (& Column Chromatography)

- Factors that influence separation & rate of elution:

  1. Polarity of mobile phase (solvent)
     - more polar solvents displace substrates from stationary phase more easily than less polar solvents (all substrates)
     - more polar the mobile phase, faster the substrate travels
     - can increase polarity to point where get no separation at all

<table>
<thead>
<tr>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Least Polar</td>
</tr>
<tr>
<td>Hexanes</td>
</tr>
<tr>
<td>Carbon Tetrachloride</td>
</tr>
<tr>
<td>Toluene</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Diethyl Ether</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>Acetic Acid</td>
</tr>
<tr>
<td>Most Polar</td>
</tr>
<tr>
<td>Water</td>
</tr>
</tbody>
</table>
Factors that Affect TLC (& Column Chromatography)

• Factors that influence separation & rate of elution:

2. Substrate interactions with stationary phase
   • stronger the interaction, more slowly the substance moves
   • polar substrates move more slowly than non-polar ones
     (polarity = ability of substance to bind to stationary phase)

<table>
<thead>
<tr>
<th>Compound Type</th>
</tr>
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<tbody>
<tr>
<td>Least Polar</td>
</tr>
<tr>
<td>Alkanes</td>
</tr>
<tr>
<td>Alkenes</td>
</tr>
<tr>
<td>Ethers</td>
</tr>
<tr>
<td>Alkyl Halides</td>
</tr>
<tr>
<td>Aromatics</td>
</tr>
<tr>
<td>Aldehydes and Ketones</td>
</tr>
<tr>
<td>Alcohols</td>
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<tr>
<td>Amines</td>
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<tr>
<td>Organic Acids</td>
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<tr>
<td>Salts</td>
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</tbody>
</table>

Most Polar

<p>| |</p>
<table>
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<th></th>
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</thead>
</table>
Intermolecular Forces

- Influence adsorption of molecules on the stationary phase:
  
  1. Hydrogen Bonding
     - alcohols: R-OH
     - carboxylic acids: \( \text{R-C-OH} \)
  
  2. Dipole-Dipole (Electrostatic) Interactions
     - aldehydes: \( \text{R-C-H} \)
     - ketones: \( \text{R-C-R'} \)
  
  3. Coordination
     - amines: \( \text{R-NH}_2 \)
  
  4. Van der Waals interactions
     - hydrocarbons: \( \text{R-CH}_3 \)
Thin Layer Chromatography Technique

- Performed on glass or plastic plate spread with thin layer of dry adsorbent (solid phase)
- Sample spotted on plate using fine capillary tube
- Plate put into developing chamber; capillary action draws solvent (mobile phase) up the plate carrying various components with it.
- Mark solvent front with pencil; let plate dry
- Visualize & evaluate spots
Thin Layer Chromatography

• Visualization

  colored compounds - just look!

  colorless compounds
  1. UV light - fluorescent indicator in adsorbent
     dark spots against a bright background
  2. Iodine chamber
     I$_2$ adds reversibly to many compounds
     brown spots against a yellow background
  3. Chemical Stain
     many possibilities
     typically destructive

not permanent, mark with pencil
Calculating \( R_f \) Values

- TLC Data can be quantified using "ratio to front" or \( R_f \) values

\[
R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}
\]

more polar compounds = small \( R_f \)
less polar compounds = large \( R_f \)

(measure from baseline to center of spot)
**Thin Layer Chromatography: Utility**

- **Evaluation of Reaction Mixtures (can monitor reaction progress)**
  
  disappearance of one spot (starting material) & the appearance of a different spot over time indicates that the original compound has been converted to something else.

  - A: starting material
  - B: reaction mixture after 10 minutes
  - C: reaction mixture after 2 hours

- **As an Indicator of Purity**

  a pure compound should appear as a single spot by TLC; two or more spots in a single lane indicate the compound is impure.

  Careful! just because you see one spot doesn't mean the compound is pure.

  - TLC of an impure compound
  - TLC of column chromatography fractions
Thin Layer Chromatography: Utility

- Preliminary Identification of Compounds

For a given set of conditions (solvent system, adsorbent):
- two compounds having different $R_f$ values are different
- two compounds having identical $R_f$ values may be the same

CAUTION! TLC does not provide quantitative information about reaction yields or compound identity
Next Week

Experiment 1: Thin Layer Chromatography

A. Understanding $R_f$ Values
   evaluate how $R_f$ varies with length of TLC plate

B. $R_f$ values & solvent polarity
   evaluate how solvent polarity affects $R_f$ value of single compound

C. $R_f$ values & compound functionality
   evaluate how $R_f$ is affected by different functional groups

D. Identification of commercial food dye components
   investigate the make up of food coloring

Remember:
- Complete the pre-lab before you arrive (notebook)
- Dress appropriately
- Have a plan
Strategy

You must complete this experiment in the allotted time period.

• Come Prepared!
• Run through the entire experiment before repeating any parts.
  (ideally there will be no need to do so)
• Share developing chambers

A. Understanding $R_f$ values
B. $R_f$ values & solvent polarity

Do one of these experiments first!

TECHNIQUE IS IMPORTANT!!
practice spotting sample on spare TLC plate
GOAL: small, compact spots

C. $R_f$ values & compound functionality
D. Identification of commercial food dye components - will take the longest
Some Pointers:

⇒ Spotting the Plate

- Small, compact spots give best results by TLC
  don't overload the plate - will get streaking
  practice first!!
- Use only pencil when drawing on TLC plates
  ink may run!
- Take care not to contaminate the samples!!!
  results will be meaningless

⇒ Preparing the Developing Chamber

- Assemble the components
  glassware should be clean!
  cover should be on!
- Filter paper should be saturated with solvent
  keeps atmosphere saturated w/vapors
  stops evaporation of eluent from plate
- Add about 0.5cm of solvent (about 3mL)
  level after the filter paper is saturated
Some Pointers:

- **Developing the Plate**
  - Solvent level MUST be below level of the spots so samples don't wash off
  - Don't lean plate against filter paper will get uneven elution - distorts your results
  - Remove plate before solvent reaches the top! otherwise, invalidates $R_f$ values
  - Let plates dry before visualizing with UV light or iodine

![Diagram of TLC development](image)