**Chromatography**

Chromatography: The separation of a mixture of two or more compounds or ions by distribution between two phases, one which is stationary and the other which is mobile (moving).

The basic principle depends on the different solubilities or adsorptivities of the substances to be separated relative to the stationary or the mobile phases.

The types of chromatography are routinely used in the organic chemistry teaching labs are:

- **Solid-Liquid Chromatography:** The stationary phase is solid and the mobile phase is a liquid. Examples are: column chromatography, TLC, and paper chromatography.

- **Liquid-Liquid Chromatography:** HPLC

- **Gas-Liquid Chromatography:** The stationary phase is a liquid and the mobile phase is a gas. Example: Gas Chromatography.

Let’s look at an example:
II. Variables Affecting Separations

A. Solute-stationary phase interactions

- Stationary phases:
  - The most common types of stationary phases used in organic chemistry lab are:
    - $\text{SiO}_2 \times \text{H}_2\text{O}$ (silica)
    - $\text{Al}_2\text{O}_3 \times \text{H}_2\text{O}$ (alumina) (may be acidic, basic, or neutral)
  - Polar solutes will bind more strongly to the polar stationary phase as compared to less polar solutes.
  - There are some common types of interactions to note:
    Salt formation $>$ Coordination $>$ H-Bonding $>$ Dipole-Dipole $>$ London-London

B. Solute-Solvent Interactions

- More polar solvents will remove polar solutes from stationary phase.
- For example:
  - Ketone will elute from $\text{Al}_2\text{O}_3$ with chloroform (CHCl$_3$) but not with hexane.

The goal is to choose a solvent that allows for the solutes to be separated from each other…

Specifically for Column Chromatography...

C. Size of Columns

- As a general rule $\rightarrow$ The amount of absorbent should be $\sim$ 25-30 times (by mass) the amount of material to be separated.
- The column should have a height to diameter ratio of about 8:1.

D. Flow Rate

- Too slow, diffusion may occur and separation may be compromised
- Too fast, no time to establish a good equilibrium.
III. General Concept of Column Chromatography

- The solute is loaded onto the column and an equilibrium is established between the solid phase and the liquid phase.

Solvent Flows this way down the column (Mobile Phase)

Steady flow of solvent

Fractions collected sequentially

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IV. Thin-Layer Chromatography

A. Preparing the TLC Plate
   - Gently mark the origin of your TLC plate using a pencil
   - Samples are then spotted onto the TLC plate
     - Spotting the sample requires you to lightly touch the pipet to the TLC plate.
     - Capillary action will cause the solution to fill the pipet.
     - Be careful to not spot too much (you will not get good resolution)

B. Developing the TLC Plate
   - Ideally, we want the analytes to move about 40-50% of the length of the plate.

C. Visualizing Your Results
   - Iodine (reacts with many functional groups to form brown/yellow spots)
   - Ultraviolet lamp
   - TLC plates with fluorescent indicator
   - Chemical methods
     - Ninhydrin (reacts with amino acids)

D. Monitoring a reaction via TLC...
E. Rf Values: Quantifying Your Results

- A Rf is the relative distance that a sample component has moved relative to the distance moved by the developing solvent.
- Rf is measured by dividing the distance the component traveled by the distance the solvent traveled. Therefore, an Rf value can never be greater than 1.

\[
R_f = \frac{\text{spot distance traveled}}{\text{eluent distance traveled}}
\]

- The Rf value for a particular component is characteristic for that component in that particular solvent.
- Therefore, it will always be the same (considering that the mobile and stationary phases are the same) and can be identified in other mixtures.

Example TLC Plate

<table>
<thead>
<tr>
<th>Solute</th>
<th>Distance Traveled (cm)</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.0</td>
<td>2.0 cm = 0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0 cm</td>
</tr>
<tr>
<td>B</td>
<td>3.0</td>
<td>3.0 cm = 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0 cm</td>
</tr>
<tr>
<td>C</td>
<td>3.5</td>
<td>3.5 cm = 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0 cm</td>
</tr>
<tr>
<td>D</td>
<td>5.5</td>
<td>5.5 cm = 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0 cm</td>
</tr>
</tbody>
</table>