# *Experiment 1: Thin Layer Chromatography*

Part A: understanding $R_f$ values

Part B: $R_f$ values & solvent polarity

Part C: $R_f$ 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 $Al_2O_3$
  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

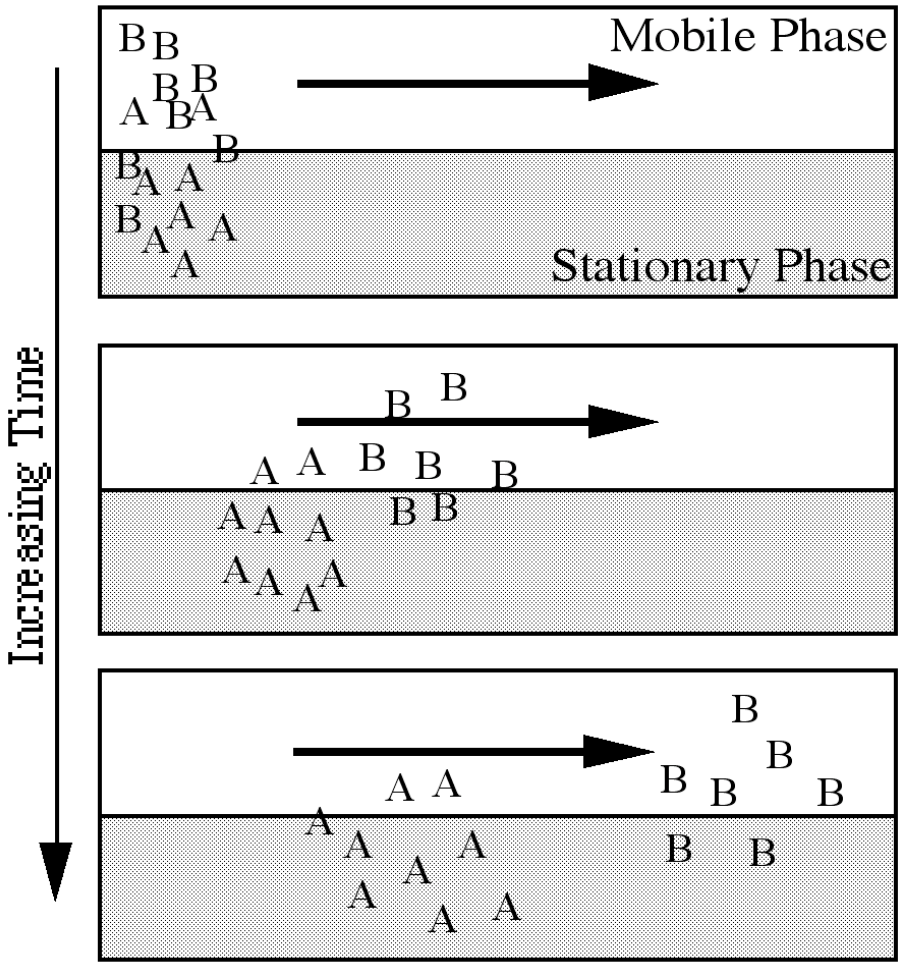
  - adsorption depends on interaction of specific component with stationary phase
  - stronger interaction = more molecules adsorbed on stationary phase, less in mobile phase

- Effectively establish an equilibrium for each component:


- Differences in equilibrium allow separation

# *Chromatographic Separation*

- Consider a 2-component mixture (A + B):



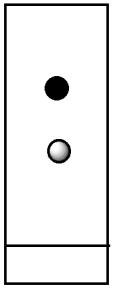

- establish equilibrium
- adsorption A >> B

- more B in mobile phase
- ∴ B moves faster than A

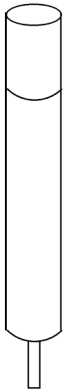
- component separation increases with distance mobile phase travels

# *Types of Chromatography*

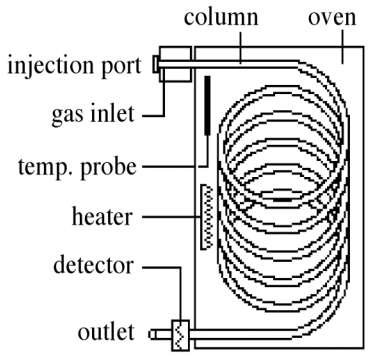
➔ 1. Thin Layer Chromatography (TLC)
- stationary phase: spread over glass or plastic sheet
- mobile phase: liquid; drawn up plate by capillary action



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:

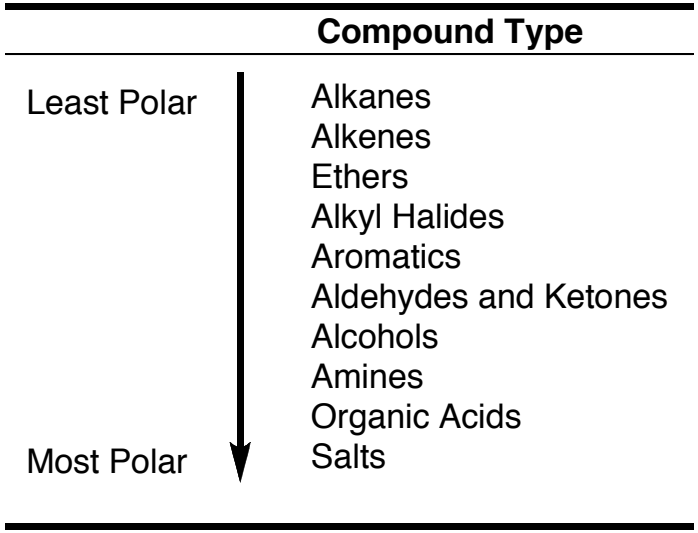
1. Polarity of mobile phase (solvent)
   - more polar solvents displace substrates from stationary phase more easily than less polar solvents (*all substrates*)
   - more polar the mobile phase, faster the substrate travels
   - can increase polarity to point where get no separation at all

| | Solvent |
|---|---|
| Least Polar | Hexanes |
| | Carbon Tetrachloride |
| | Toluene |
| | Chloroform |
| | Diethyl Ether |
| | Ethyl Acetate |
| | Acetone |
| | Methanol |
| | Acetic Acid |
| Most Polar | Water |

# *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)

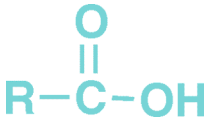


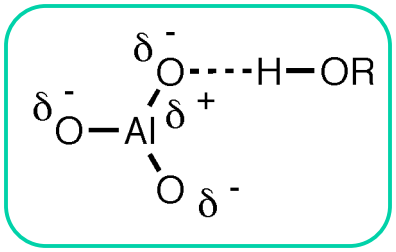
# *Intermolecular Forces*

- Influence adsorption of molecules on the stationary phase:

1. Hydrogen Bonding

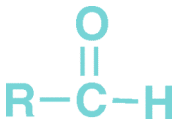
   alcohols R-OH

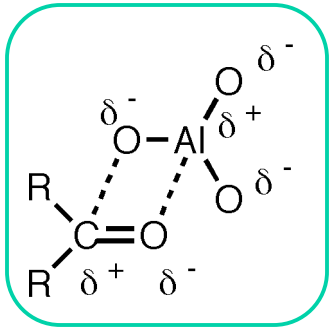
   carboxylic acids 




2. Dipole-Dipole (Electrostatic) Interactions

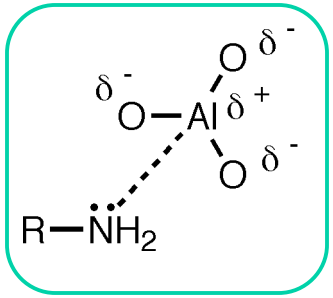
   aldehydes

   ketones  




3. Coordination

   amines R-$NH_2$

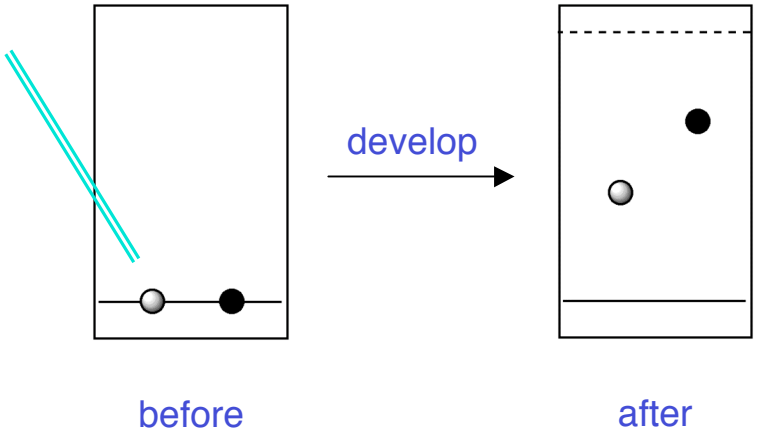


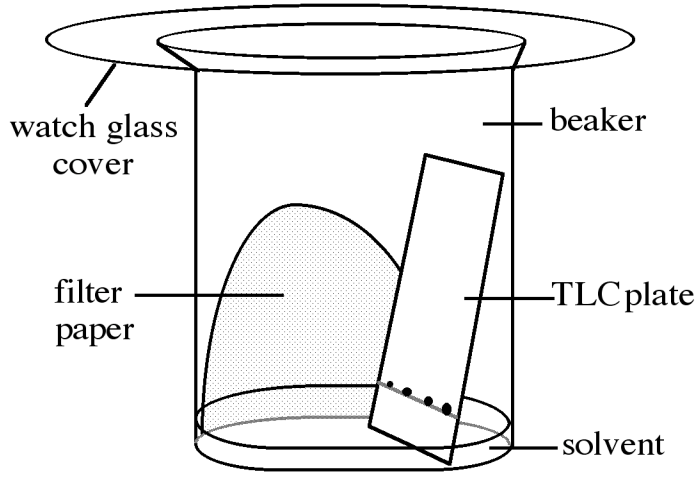

4. Van der Waals interactions

   hydrocarbons 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

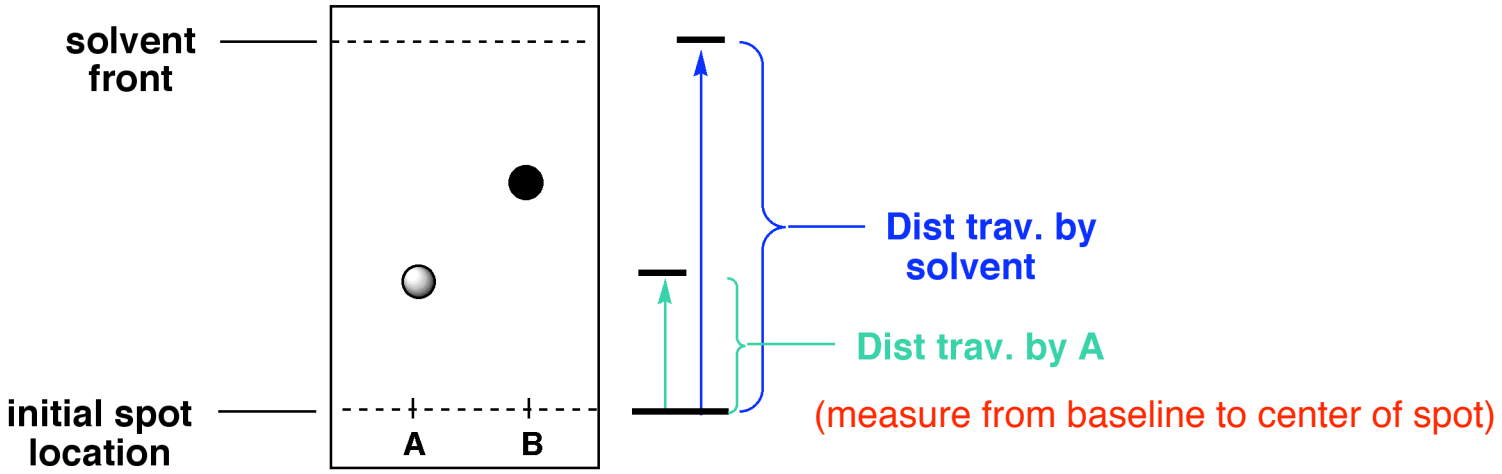
  1. UV light - fluorescent indicator in adsorbent
     dark spots against a bright background
  2. Iodine chamber
     $I_2$ adds reversibly to many compounds
     brown spots against a yellow background
  3. Chemical Stain
     many possibilities
     typically destructive

not permanent, mark with pencil

# *Calculating Rf Values*

- TLC Data can be quantified using "ratio to front" or $R_f$ values

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

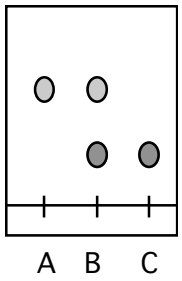



more polar compounds = small $R_f$
less polar compounds = large $R_f$

# *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.

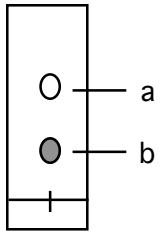



A: starting material
B: reaction mixture after 10 minutes
C: reaction mixture after 2 hours

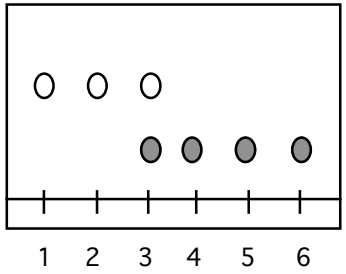
- As an Indicator of Purity

  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



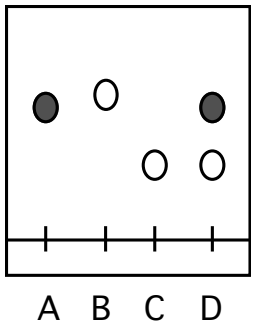

TLC of column chromatography fractions

# *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




A-C: known components
D: unknown mixture

**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!!

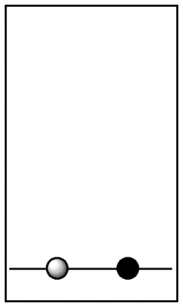
practice spotting sample on spare TLC plate

GOAL: small, compact spots

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

# *Some Pointers:*

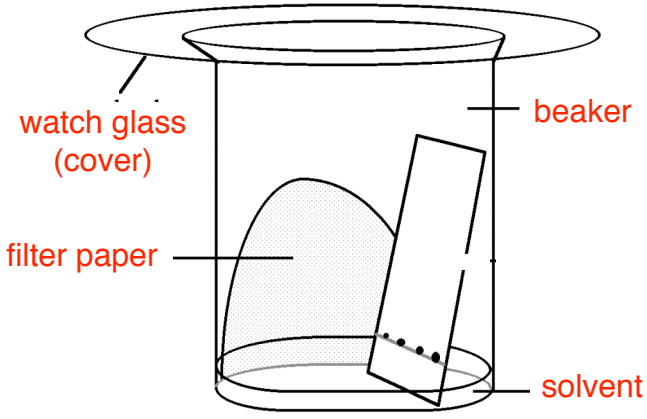
## ➔ Spotting the Plate



- 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

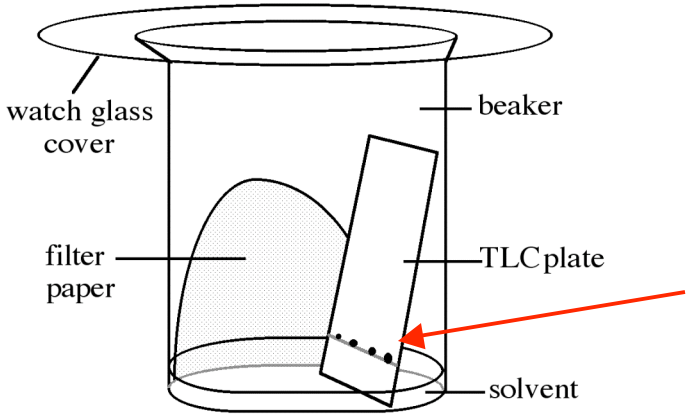
- Assemble the components
  - glassware should be clean!
  - cover should be on!
- Filter paper should be saturated with solvent
  - keeps atmosphere saturated w/vapors
  - stops evaporation of eluent from plate
- Add about 0.5cm of solvent (about 3mL)
  - level <u>after</u> the filter paper is saturated



# *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




**note level of spots!**
(above the eluent)