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

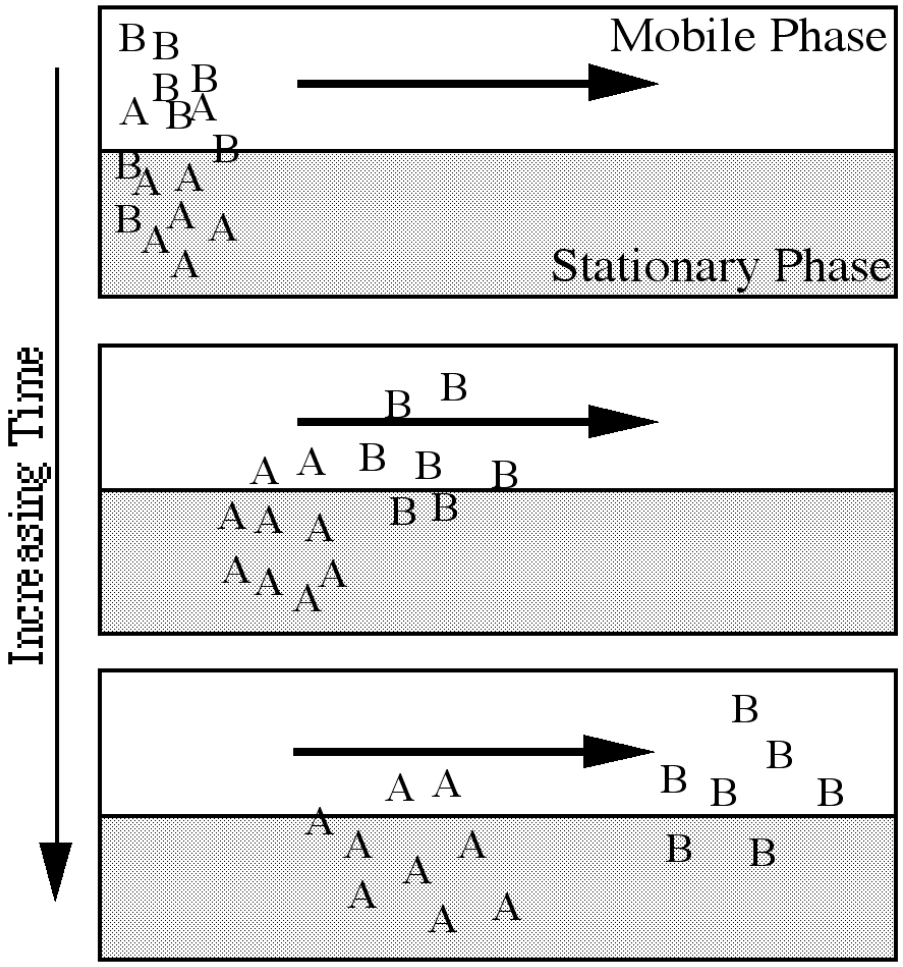
  - adsorption depends on interaction of specific component with stationary phase
  - stronger interaction = more molecules adsorbed on stationary phase, less in mobile phase

- Effectively establish an equilibrium for each component:

- Differences in equilibrium allow separation

Chromatographic Separation

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



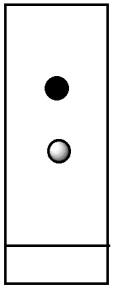
- establish equilibrium
- adsorption A >> B

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

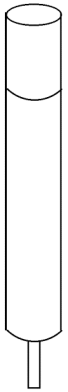
- component separation increases with distance mobile phase travels

Types of Chromatography

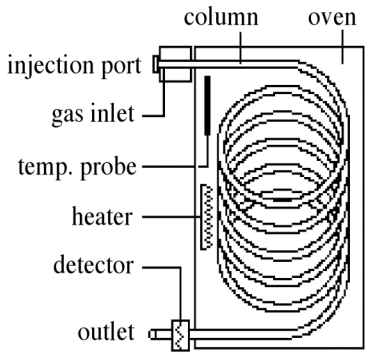
➔ 1. Thin Layer Chromatography (TLC)
- stationary phase: spread over glass or plastic sheet
- mobile phase: liquid; drawn up plate by capillary action



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:

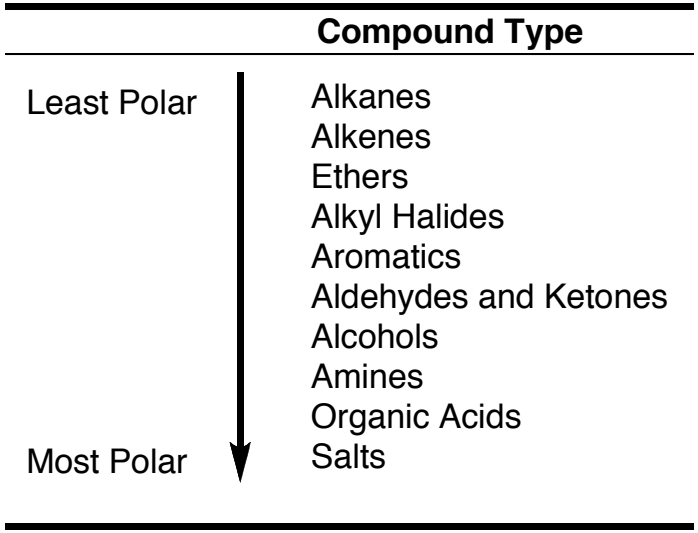
1. Polarity of mobile phase (solvent)
   - more polar solvents displace substrates from stationary phase more easily than less polar solvents (*all substrates*)
   - more polar the mobile phase, faster the substrate travels
   - can increase polarity to point where get no separation at all

	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)

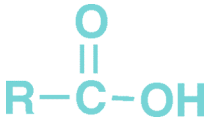


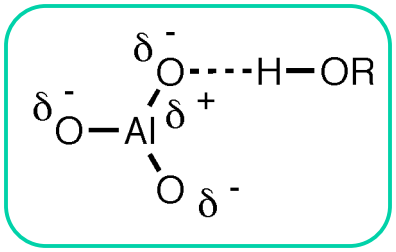
Intermolecular Forces

- Influence adsorption of molecules on the stationary phase:

1. Hydrogen Bonding

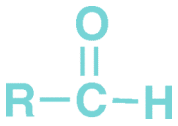
   alcohols R-OH

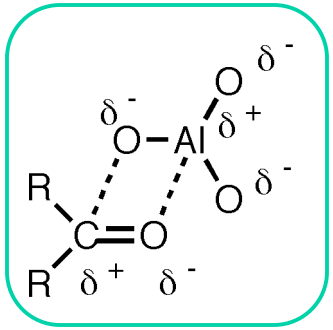
   carboxylic acids 



2. Dipole-Dipole (Electrostatic) Interactions

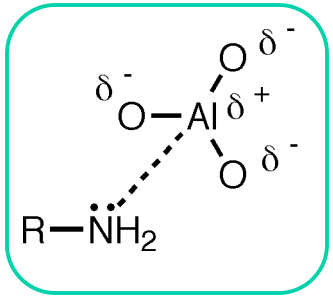
   aldehydes

   ketones  



3. Coordination

   amines R-NH_2

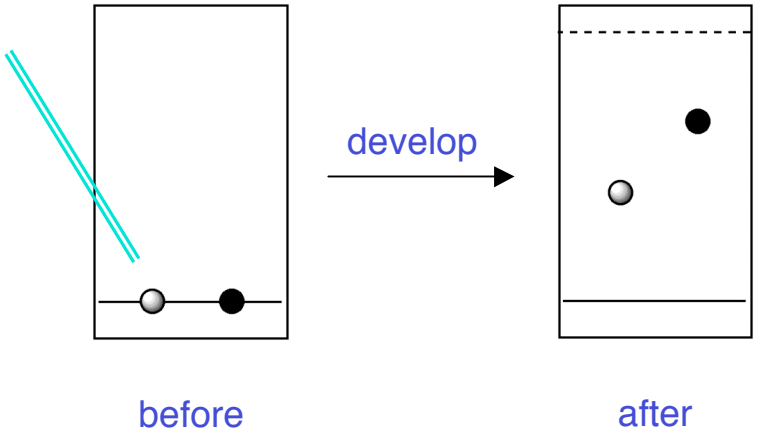


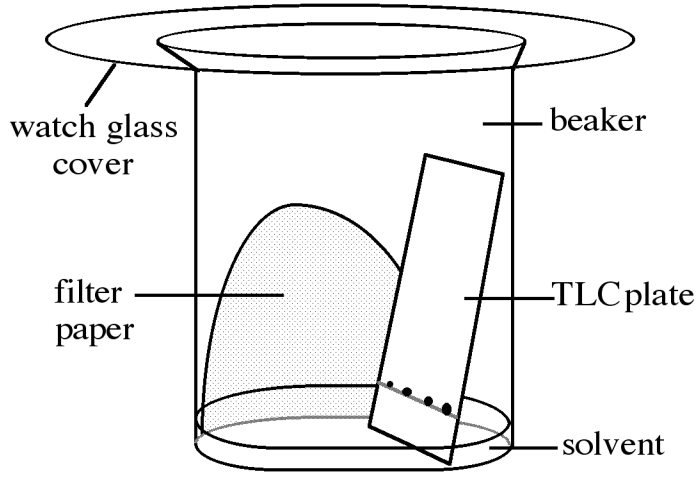
4. Van der Waals interactions

   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

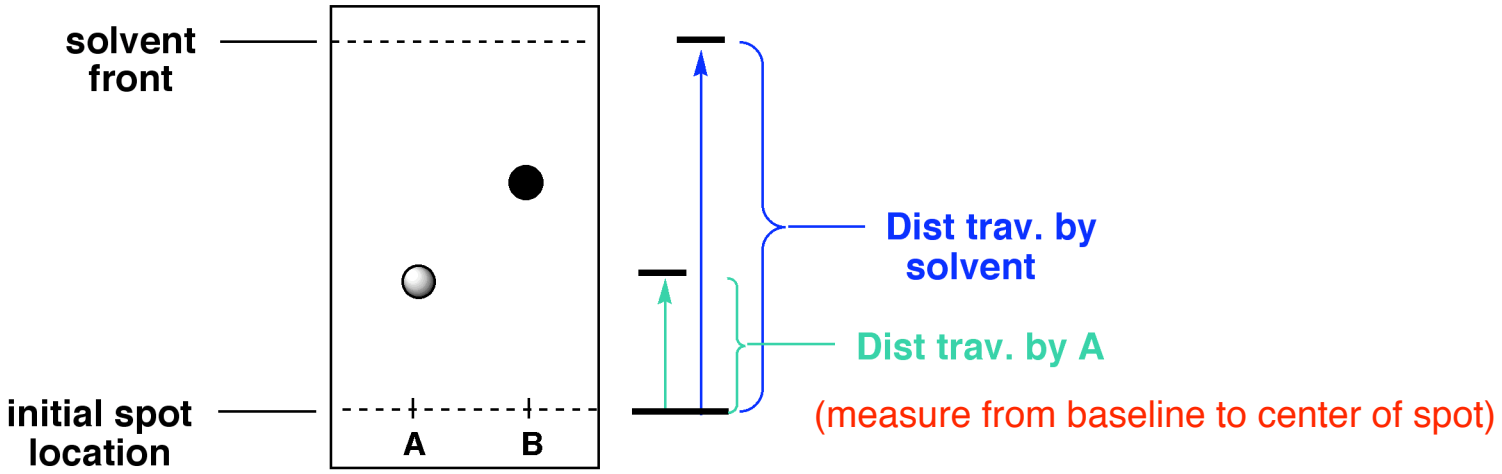
  1. UV light - fluorescent indicator in adsorbent
     dark spots against a bright background
  2. Iodine chamber
     I_2 adds reversibly to many compounds
     brown spots against a yellow background
  3. Chemical Stain
     many possibilities
     typically destructive

not permanent, mark with pencil

Calculating Rf Values

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

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

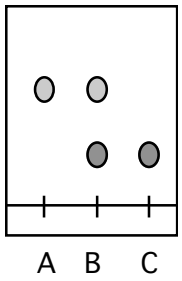


more polar compounds = small R_f
less polar compounds = large R_f

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.

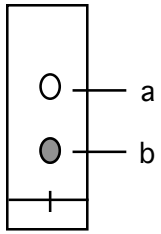


A: starting material
B: reaction mixture after 10 minutes
C: reaction mixture after 2 hours

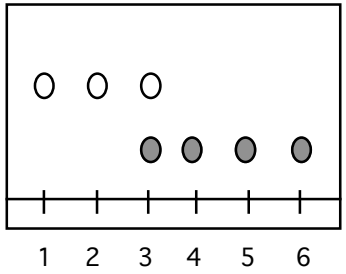
- As an Indicator of Purity

  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



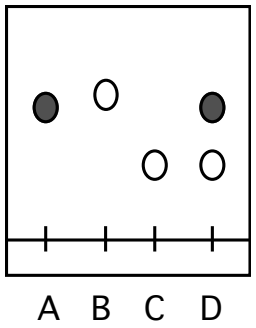
TLC of column chromatography fractions

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!!

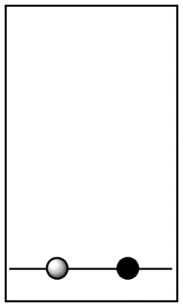
practice spotting sample on spare TLC plate

GOAL: small, compact spots

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

Some Pointers:

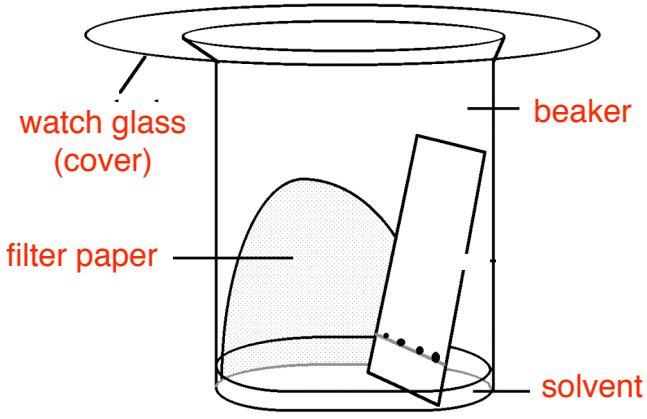
➔ Spotting the Plate



- 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

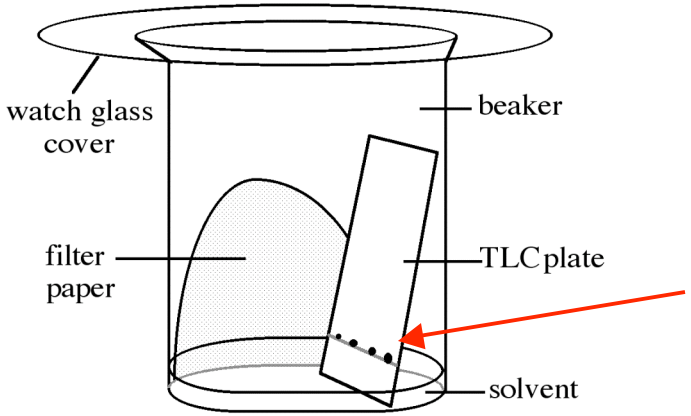
- Assemble the components
  - glassware should be clean!
  - cover should be on!
- Filter paper should be saturated with solvent
  - keeps atmosphere saturated w/vapors
  - stops evaporation of eluent from plate
- Add about 0.5cm of solvent (about 3mL)
  - level <u>after</u> the filter paper is saturated



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)