# II Chromatography

- most techniques that we will be studying require pure compounds, and therefore require a separation step prior to analysis
- you have all seen thin-layer chromatography (TLC) and gas chromatography (GC) in organic lab
- all chromatographic separations require
  - two phases:
    - mobile phase (gas or liquid)
    - stationary phase (solid or liquid)
  - phase boundary between the phases- across which analytes partition
  - separation increased by moving one phase with respect to the other

# II. Chromatography

- A. <u>principles of chromatography</u>

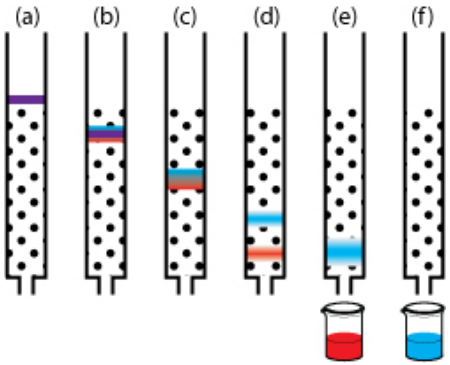
- equilibrium established between molecule in stat. and mobile phase
  - $X_m \rightleftharpoons X_s$
  - define: distribution coefficient ($K_c$) = $\frac{[X_s]}{[X_m]} = \frac{C_s}{C_m}$
  - $K_c > 1$ means:
    - more sample in stationary phase –or-
    - individual molecule spends more time in stationary phase
  - [ ] and t are related

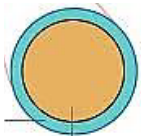
# II.A. Principles of chromatography

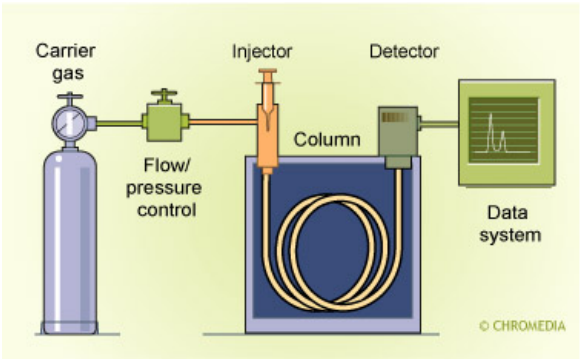
- shopping mall analogy
- two important points
  - all compounds spend some time in the column
    - $t_m$ – time for mobile phase to traverse column
  - all compounds spend the same amount of time in mobile phase
    - separation occurs in stationary phase

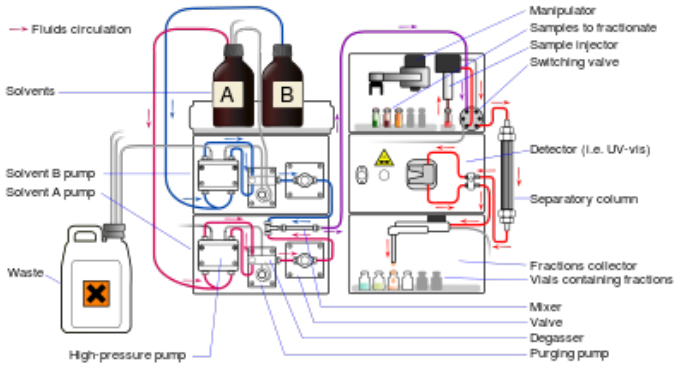
# II.B. types of chromatography

- B. <u>types of chromatography</u>
  - 1. adsorption chromatography
    - s.p. is a solid –molecules adsorb
    - m.p. is a liquid or a gas
    - examples:  TLC, column chromatography
  - 2. partition chromatography
    - s.p. is liquid on solid support analytes dissolve
    - m.p is a gas (GLC) or a liquid (LLC)
    - two types of LLC
      - normal phase – polar s.p.; non-polar m.p.
      - reverse phase – non-polar s.p.; polar m.p.



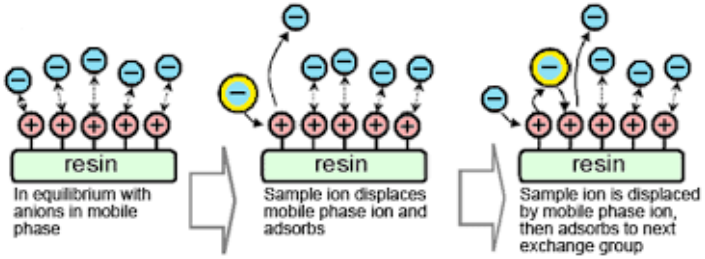





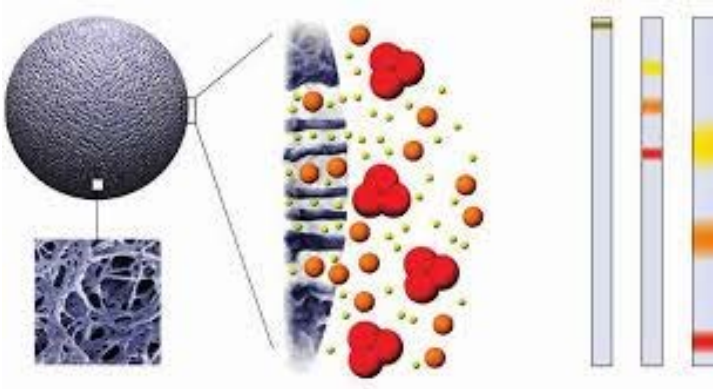



# II. B. types of chromatography

- 3. ion-exchange chromatography
  - s.p. is a resin with + or – side groups
  - m.p. is liquid
  - useful for separating ions

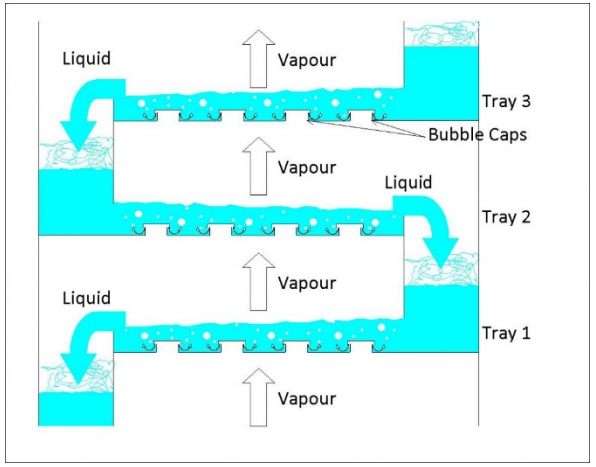


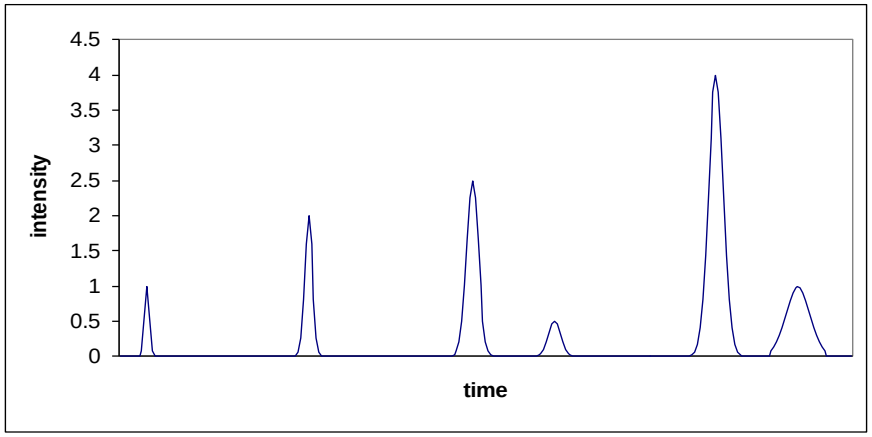

- 4. size-exclusion chromatography
  - s.p. is polymer with pores of a certain size
  - small analytes can get into pores
  - large analytes are excluded
  - m.p. is a liquid
  - separation based roughly on molecular weight

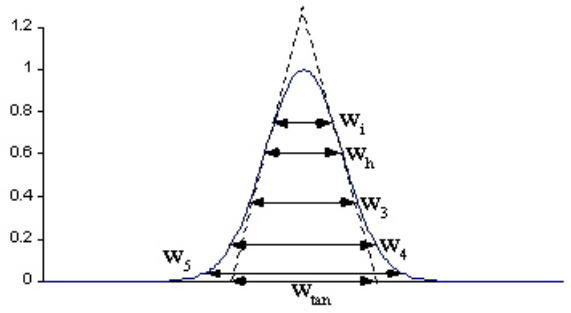


# II. C. Plate Theory

- C. plate theory
- 1. theoretical plates (N)
  - comes from plate distillations
  - more plates = better separation
  - $N = 16\left(\frac{t_R}{w_b}\right)^2$
    - $t_R$ is retention time
    - $w_b$ is width of peak at baseline



# II. C. Plate Theory

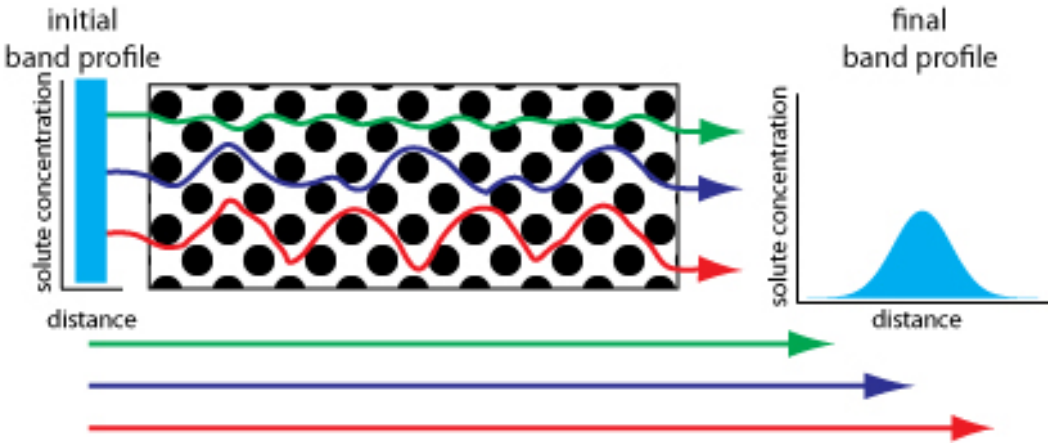
- 2. Height equivalent to a theoretical plate (HETP, H)
  - since N will depend on the length of the column, we need a more comparable metric of how efficient the separation is
    - increase L, N will get bigger
    - BUT!!!! as we will see on the next slide, the widths of the peaks will also increase with increasing time spent in the column
  - H = L/N
    - normalizes N for length of column
    - smaller H means a more efficient separation
    - dependent on:
      - technique – GC/LC/CE etc
      - choice of mobile and stationary phases
      - temperature
      - flow rate

# II. D. Rate theory

- D. rate theory
  - H can be related to physical properties involved in the separation process
  - Since H depends on N, and ultimately the broadening of the peaks (w), we must look into the causes of peak broadening.
  - The Van Deemter equation covers all of this.
  - $H = A + \frac{B}{\bar{u}} + C\bar{u}$
    - $\bar{u}$ is the average linear velocity (cm/s)
    - can be related to flow rate ($cm^3/s$) through cross sectional area of the column ($\sigma$, $cm^2$)
    - developed for packed GC columns but easily extended to other techniques

# II. D. Rate theory

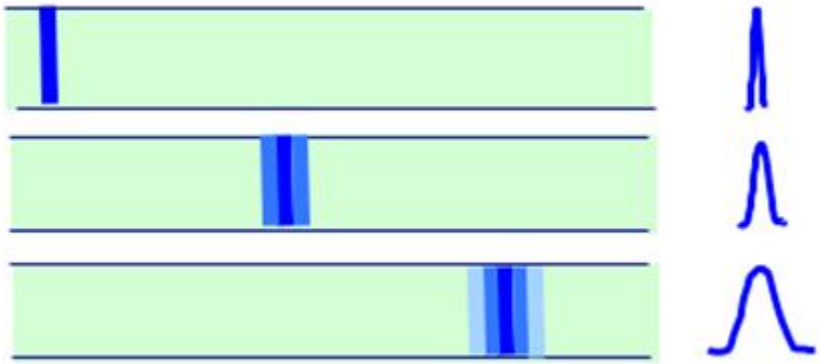
- $H = A + \frac{B}{\bar{u}} + C\bar{u}$
- A accounts for "eddy" diffusion
  - arises from different paths through the packing material




  - depends on size (diameter) of the packing particle ($d_p$)
  - $A = 2\lambda d_p$
  - l is a constant related to packing material (0 – 1)
  - eddy diffusion is <u>independent</u> of flow velocity

# II. D. Rate theory

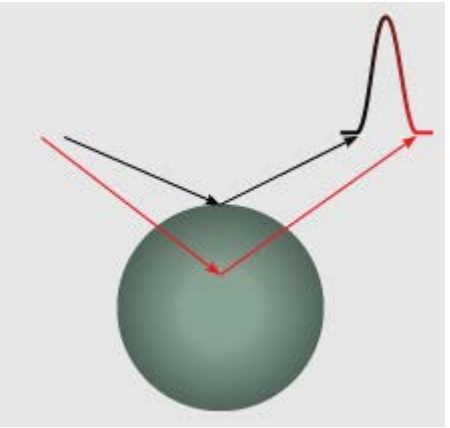
- $H = A + \frac{B}{\bar{u}} + C\bar{u}$
- B arises from longitudinal diffusion
  - analyte is injected as a "plug"
  - this creates a concentration gradient in front and behind plug



  - depends on diffusion constant of analyte in mobile phase ($D_m$)
  - $B = 2\gamma D_m$ where, $\gamma$ is an obstruction factor
  - <u>inversely</u> proportional to flow velocity

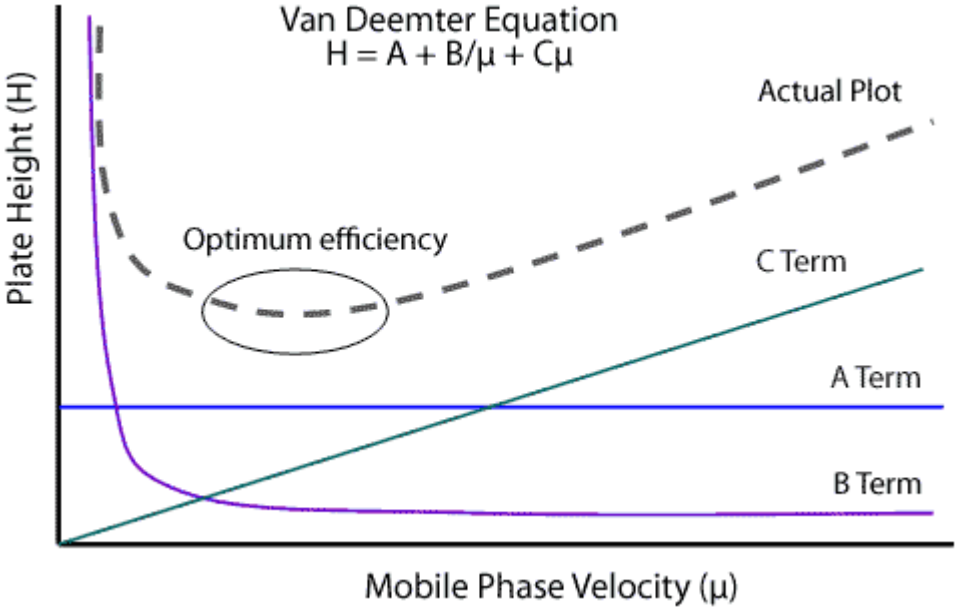
# II. D. Rate Theory

- $H = A + \frac{B}{\bar{u}} + C\bar{u}$
- C arises from mass-transfer broadening
  - recall that the s.p. consists of a liquid layer on solid support
  - some analytes penetrate to greater depth into the liquid layer
  - depends on size of layer (d of particle)
  - depends on diffusion in stationary phase
  - $C = \frac{1}{6}\frac{d_p^2}{D_m}$
  - proportional to flow rate



# II. D. Rate Theory

- $H = A + \frac{B}{\bar{u}} + C\bar{u}$
- since you have one term that is proportional to flow rate and one term that is inversely proportional to flow rate, there will be an optimal flow rate that produces the minimum H



# II. E. Retention factors and resolution

- E. Retention factors and resolution
  - if two different compounds have different interactions with s.p., they will separate
  - define: retention factor (k)
    - $k = \frac{t_R - t_m}{t_m} = \frac{t'_R}{t_m}$ also $k = \frac{V_R - V_m}{V_m}$
    - gives and indication of separability of compounds
  - ultimately, want to resolve different compounds.
  - define: resolution ($R_s$)
    - $R_s = \frac{t_{R,2} - t_{R,1}}{{}^1/_2(w_2 + w_1)}$
    - $R_s$ = 0.6: can discern a valley between two peaks
    - $R_s$ = 1.5: baseline resolved

  -or-

  - -define: selectivity factor or separation factor ($\alpha$)
    - $\alpha = \frac{t'_{R,2}}{t'_{R,1}} = \frac{k_2}{k_1}$

# II. E. Retention factors and resolution

- mathematical example.
  - Given L = 15 cm,
  - calculate N, H, $k_A$, $k_B$, $R_s$, $\alpha$

| compound | $t_r$(min) | w (min) |
|---|---|---|
| solvent | 1.5 | --- |
| A | 10.2 | 0.8 |
| B | 11.1 | 0.88 |