Liquid Chromatography

Chromatography:
Processes which allow the resolution of solute mixtures by selective fixation and liberation on a solid support with the aid of directional fluid flow

Mikhail Tswett, 1901-1906
- Separation of plant pigments on CaCO₃ and alumina powder
- Tswett = Color . . .

Color Writing….?
Thin Layer Chromatography

- Stationary Phase
  - Thin layer of particles adhered to plate

- Mobile Phase
  - Solvent in bottom of tank/reservoir
  - Driven up plate by capillary action

- Separation is driven by partitioning between stationary and mobile phases

TLC Apparatus
Column Chromatography

- Stationary phase is packed into a column
- Fluid flow is driven by pumping or gravity
- Same basic biophysical principles underlie both column and thin layer chromatography

Chromatography Flavors

- Partition Chromatography
  - “Normal” phase
  - Reverse phase
- Ion Exchange Chromatography
  - Anion exchange
  - Cation exchange
- Size Exclusion Chromatography
- Affinity Chromatography
- Solid Phase Extraction
Chromatography Equations

- Equilibrium partition ratio, $K_x = \frac{[X]_s}{[X]_m}$
  - $\left(\frac{\text{Amt } X_s}{\text{Amt } X_m}\right) \times \left(\frac{V_m}{V_s}\right)$
  - $k' \times \beta = \text{‘capacity factor’} \times \text{‘phase ratio’}$

- Capacity factor, $k' = \frac{(t_r - t_m)}{t_m}$ or $\frac{t'_r}{t_m}$
  - $t_r = \text{retention time}, t'_r = \text{adjusted retention time}$
  - $t_m = \text{mobile phase travel time}$

- Phase ratio, $\beta = \frac{r_c}{2d_f}$
  - $r_c = \text{column radius}$
  - $d_f = \text{film thickness}$

Retention Time

Chromatography Equations

- Selectivity, \( \alpha = \frac{K_B}{K_A} = \frac{(t'_r)_B}{(t'_r)_A} \)
  - Ratio of partition constants of two components
  - The amount by which two components can be separated in a given mobile phase
  - Different for different stationary phases

- Resolution, \( R_s = \frac{2d}{[(w_b)_A + (w_b)_B]} \)
  - \( d \) = distance of peak separation
  - \( w_b \) = peak width at the base
  - Measure of column efficiency

Theoretical Plate Model

- Adapted to chromatography by A.J.P. Martin and R.L.M. Synge in 1941

- Based on fractional distillation theory
  - Series of sequential, equilibrium partitioning
  - Actually continuous, not-quite-equilibrium

- \( N \): Number of Theoretical Plates

- \( H \): Height Equivalent to a Theoretical Plate
Number of Theoretical Plates

- \( N = 16\left(\frac{t_r}{w_b}\right)^2 = 5.54\left(\frac{t_r}{w_h}\right)^2 \)
  - \( w_b \) = base peak width
  - \( w_h \) = peak width at half height
- \( R_s = \left(\frac{\sqrt{N}}{4}\right)(\alpha - 1)(k'/1 + k') \)
  - \( k' \) here is the average capacity factor for two closely eluting peaks
- Both \( N \) and \( R_s \) are measures of efficiency
  - \( N \) does not require two peaks
  - \( N \) is independent of relative selectivity

Height Equivalent to a T. P.

- \( H = \frac{L}{N} \)
  - \( L \) = length of the column
  - Assigns an arbitrary ‘height’ based on physical length of (vertical) column
- Those Dutch…..
  - Van Deempter Equation, the ABCs of \( H \)
  - Describe how column variables affect \( H \) [\( N \)]
  - \( H = A + B/u + Cu \)
  - \( u \) = linear mobile phase velocity
The Van Deempter Terms

A – *The Multipath Term*
- Accounts for varying particle paths
- Results in band broadening
- Constant for all mobile phase velocities

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B – *The Longitudinal Diffusion Term*
- Accounts for random walk diffusion
- Occurs in both mobile & stationary phases
- Faster the mobile phase, the less time molecules have to diffuse randomly
- Term’s effects on $H$ are inversely proportional to mobile phase velocity, $u$
The Van Deempter Terms

- **C – The Mass Transfer Term**
  - Movement between phases is not equilibrium at every point along column
  - Faster mobile phase allows less time to get closer to phase equilibrium
  - A fraction of analyte moves ahead without diffusing into stationary phase
  - A fraction of analyte lags behind in stationary phase while the bulk moves on

The Van Deempter Plot

A = 0, C_s = ‘C’ term for stationary phase, C_m = ‘C’ term for mobile phase
Broadening Due To Mixing

- Detector Mixing
  - Detector is designed for large volume/low flow
  - Column generates low volume/fast flow
  - Close peaks will resolve on column and unresolve in detector chamber

- The Dreaded Air Bubble
  - Air bubbles in the column can disrupt packed bed or leave a void upon redissolution
  - These voids become small in-column mixing chambers and result in loss of resolution

Other Peak Distorting Factors

- Sample Overload
  - Like a huge rush of people into a store on the morning of a big sale
  - Generates a leading peak shape

- Additional Retention Sites
  - A limited number of additional sites which bind sample, in addition to desired retention, will retard progress through column
  - Generates a trailing peak shape