Chromatography- Separation of mixtures
CHEM 212

What is **solvent extraction** and what is it commonly used for?

- accumulating an analyte by transfer b/w solvents
- used to isolate or concentrate analyte

How does solvent extraction work?

partitioning / accumulation / transfer of solvent between two immiscible phases.

Write the partitioning coefficient for the following reaction: $S_{phase1} \Leftrightarrow S_{phase2}$

$$K = \frac{[S]_2}{[S]_1}$$

Write the equation for calculating the fraction remaining in phase 1 after n extractions.

$$x^n = \left(\frac{V_1}{V_1 + KV_2}\right)^n$$ = fraction remaining in phase 1 after n extractions

What is the distribution coefficient? How is it different from K?

D considers that there is more than one solute species in solution

ex acids either HA or A^-

Which solvent is preferred by the charged and uncharged species?

Charged Aqueous solvent

Uncharged organic solvent

Write the equation for D in terms of Ka for an acid and base solute

Base

$$D = \frac{[B]_2}{[B]_1 + [BH^+]_1} = \frac{K \cdot K_a}{K_a + [H^+]}$$

Acid

$$D = \frac{K[H^+]}{[H^+] + K_a}$$

Write the equation for D for metal extraction using a chelator in terms of equilibrium constants. Ch 11

$$D = \frac{[ML_n]_{org}}{[M^{n+}]_{aq}} \cong \frac{K_M \beta K_a^n [HL]_{aq}^n}{[H^+]_{aq}^n} = \frac{K_M \beta K_a^n}{K_L^n} \frac{[HL]_{org}^n}{[H^+]_{aq}^n}$$

K_M = ~~formation constant~~ partitioning of ML_n complex b/w aq & solvent phase

β = formation constant overall for ML_n complex

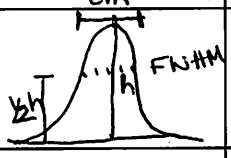
K_a = acid dissociation constant for chelator

K_L = ligand partition constant b/w aq & solvent.

Describe in general terms what chromatography does and how it works.

chromatography separates a mixture into its component constituents

Define the following terms in words and with an equation and/or units, if applicable.

Term	Symbol	Equation	Description
Retention time	t_r		time from injection for analyte to emerge from column
Adjusted retention time	t'_r	$t'_r = t_r - t_m$	time to analyte elution minus the time for mobile phase elution
Volume flow rate	U_v	mL/min	rate of solvent flow through column
Linear flow rate	U_x	cm/min	rate of solvent flow through column
Relative retention	α	$\alpha = \frac{t'_{r_2}}{t'_{r_1}} > 1$	or separation factor describes peak separation
Partition coefficient	K	$K = \frac{C_s}{C_m}$	the concentration of analyte in stationary & mobile phases $k = K \frac{V_s}{V_m}$
retention factor	k	$k = \frac{t_r - t_m}{t_m}$ $k = \frac{t'_r}{t_m}$	time in solid phase vs time in stationary phase
Peak width FWHM	$W_{1/2}$		width of the peak measured at half of the total peak height. used because its easy to measure
Resolution	R_s	$R = \frac{0.589 \Delta t_r}{W_{1/2\,ave}}$	Resolution between peaks

Define the retention factor in words.

k is the ratio of time solutes spend in the stationary versus mobile phases

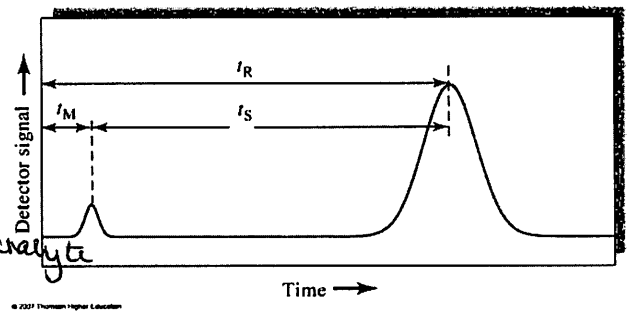
Relate the retention time to the partition coefficient

$$k = \frac{n_s}{n_m} = \frac{C_s V_s}{C_m V_m} = K \frac{V_s}{V_m}$$

Relate relative retention with the partition coefficients

$$\alpha = \frac{t'_{r_2}}{t'_{r_1}} = \frac{k_2}{k_1} = \frac{K_2}{K_1}$$

Describe the relationship between t_r, t_m, and t_r' with respect to the interaction of the mobile phase, stationary phase, and solute. Label t_r, t_m, and t_r' in the diagram below.



t_m = time from injection to mobile phase elution

t_r = total time to elution of analyte

t_r' = adjusted retention time = $t_r - t_m$

Describe the basis of separation for each of the types of chromatography described below.

Absorption Chromatography

solid stationary phase, liquid or gas mobile phase

solutes absorbed onto surface of solid particles

Partitioning Chromatography

immobile liquid stationary phase bonded to a surface

gas mobile phase

solute equilibrates b/w liq & gas mobile phase

Ion Chromatography

anions or cations are covalently bonded to a solid stationary phase resin.

liquid mobile phase

Solutes of opposite charge are attracted to the stationary phase

Size Exclusion Chromatography

Separates molecules on the basis of size. porous solid matrix

Larger molecules elute faster b/c they are restricted to "superhighways" and are not able to take a more tortous route.

Affinity Chromatography

Most selective

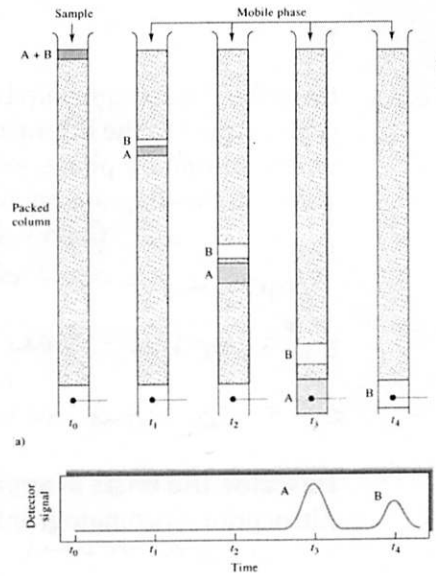
specific, strong interactions b/w solute & molecules

covalently bound to solid phase

eg. Antibody - protein

Chromatography- Separation of mixtures

1. Sample dissolved in mobile phase
2. Mobile phase passes over stationary phase
3. Analyte molecules partition between mobile and stationary phase
4. Different solutes will elute at different times

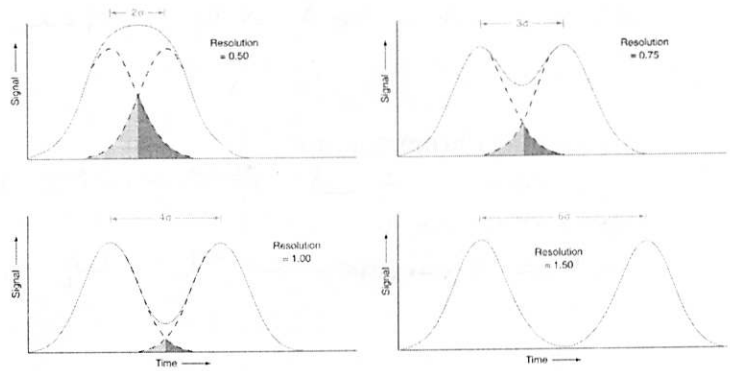


Separation of mixtures

Resolution of separations

State how resolution is calculated?

$R = \frac{0.589\,\Delta t_r}{W_{1/2}\ \text{ave}}$ ← average FWHM

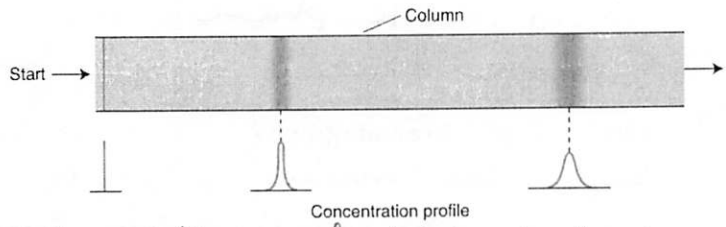


What is considered quantitative separation?

R > 1.5 is considered a quantitative separation

Better separations require either:

1. increase in rate of separations
2. decrease in rate of band spreading



Define diffusion in words. Why does it happen?

net transport of solute from regions of high to low concentration caused by random movement of molecules (or Brownian motion)

What is the relationship between bandwidth and retention time?

retention time ↑, bandwidth ↑

$$\text{flux} \cdot \frac{mol}{m^2\, s} \equiv J = -D \frac{dc}{dx}$$

D = diffusion coefficient
C = concentration
x = distance

What does a theoretical plate represent?
the distance of column needed for each solute molecule to interact with the stationary phase exactly one time

How is plate height calculated? State the equation and define each variable

$$H = \frac{\sigma^2}{x}$$

σ = standard deviation of band
x = distance traveled on column

$$\sigma = \sqrt{2Dt}$$

t = time
D = diffusion coefficient ~ 10^{-9} $\frac{m^2}{s}$

$$N = \frac{L}{H}$$

N = # theoretical plates
L = length of column

State the equation for calculating the number of plates using full width half max ($w_{1/2}$)

$$N = \frac{5.55\, t_r^2}{w_{1/2}^2}$$

What is the relationship between number of plates & separations

N ↑ , R ↑

What factors affect resolution and how are they each related to resolution?

N = # theoretical plates
L = column length

$R \propto \sqrt{N}$ $\quad R \propto \sqrt{L}$

$$R = \frac{\sqrt{N}}{4}(\gamma - 1) \qquad \gamma = \frac{t_{r2}}{t_{r1}}$$

Comparing chromatographic techniques

	TLC	GC	HPLC
Stationary phase	Silica gel on glass plate	Capillary with liquid or solid phase	Silica beads- often derivitized with C-18, etc
Mobile phase	solvent	Gas, usually He	Organic or polar solvent or mixture of solvents
Column length	~10cm	15-100m	~5-25 cm
Pressure	ambient	low	Up to 1,-000 bar
Temperature	ambient	variable	Variable

High Performance Liquid Chromatography (HPLC)

Draw an HPLC instrument diagram:

HPLC pressure- up to 1,000 bar (~100-400 bar normal)
Column length ~10-25 cm with ~5um C-18 derivativized silica particles
Solvent:
Isocratic elution

Gradient elution

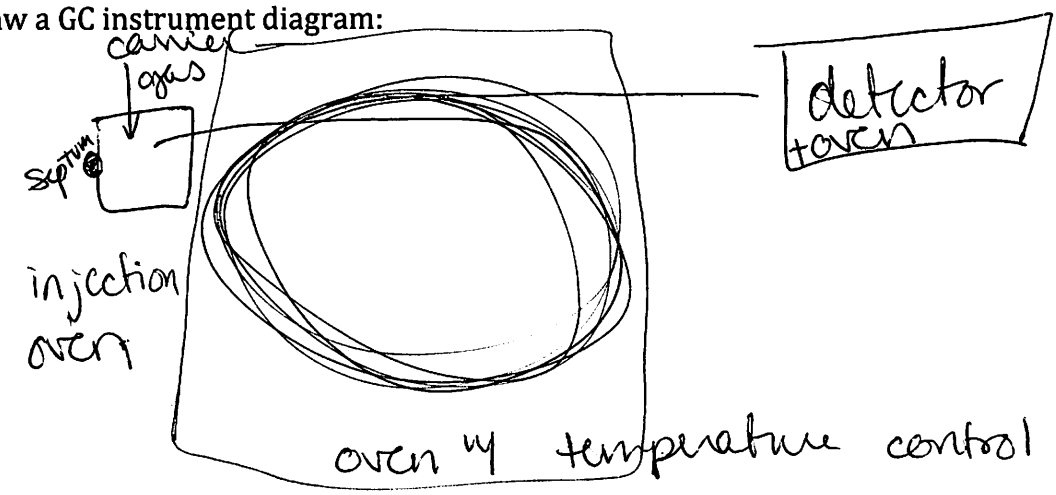
Detectors:
UV-Vis

ELSD

Mass Spectrometry

Gas Chromatography (GC)

Draw a GC instrument diagram:



State the function of each of the components of a GC:

Carrier gas He, H_2, N_2

Sample injection

Headspace injection-
sampling gas above heated solid sample

Liquid injection-
injecting analyte into instrument in a liquid solvent matrix

Solid phase microextraction (SPME)-
concentrating sample on a fiber prior to injecting fiber into instrument.

Injector chamber-
Heated chamber where solvent & analyte are volatalized. split, splitless, & on column injection

Column-
thin, open tubular column w/ stationary phase bonded to the inside of the column

0.1 - 0.53 mm diameter

Stationary phase 0.1 - 5 μm thick

Polyimide + silica capillary + stationary phase

Detector options-

thermal conductivity detector

UAF { Flame ionization detector
Mass spectrometry

Electron capture

many others

High Performance Liquid Chromatography (HPLC)

Draw an HPLC instrument diagram:

solvent reservior
↓
pump
↓
sample injector → HPLC column → detector

Solvent-

Isocratic elution

- one solvent or solvent mixture that does not vary as a function of time

Gradient elution

- solvent mixture changes as a function of time.

Column-

packed column
w/ small 1-5 μm silica particles (that may be derivatized)
column length 25-5 cm

<u>General elution problem</u> - for a complex mixture, isocratic conditions do not produce adequate separation of early & late eluting peaks simultaneously.

Detectors-

UV-Vis

UV-Vis spectra can be monitored constantly using a single beam photodiode array instrument.

ELSD Evaporative Light scattering detector

sample is nebulized, solvent evaporated, & light dispersion (affected by analyte) measured as a function of time.

Mass Spectrometry -