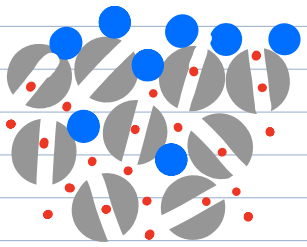
Chromatography

Stationary phase
subst. that supports mixture + allows compounds to be retained

Mobile Phase
fluid carrying compounds to be separated
- the more a compound likes the stationary phase, the longer it stays there
- ones that dont ♡ stationary will pass through

affinity for mobile = move fast
affinity for stationary = sloooow

>Size exclusion chromatography



large compounds out first
small compounds out last (more places to get stuck)

elute (leave) first = shorter retention time

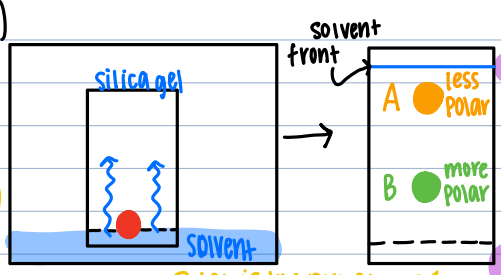
>TLC (Polarity)

separate based on polarity
sm. amounts of solids / high bp liquids (oils)

Stationary
silica gel
polar!!
can Hbond to some compounds

Mobile Phase
shallow solvent in sealable chamber

used 4 info. gathering, not rly separating



NONPOLAR comp. go further
POLAR comp dont go as far

$R_f = \frac{\text{spot}}{\text{solvent front}}$
- never >1 or (-)
- never equal for 2 comp

What is polar?
hydrocarbons
ketone/ester/alkylhalides
alcohol, carboxy. acid, amine

high Rf ↓ low Rf

rxn monitoring

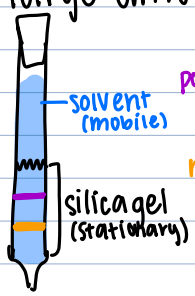
Polar is lower + slower
nonpolar = high Rf
polar = low Rf
H-bond = lowest

Polar is slower + lower

Column Chromnography - Polarity

HIGH YEILD

separate based on polarity
large amounts of solid / high bp liquids



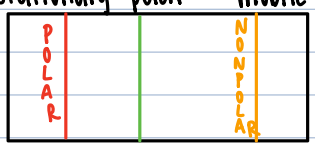
polar compound remains in column longer (elute last
nonpolar compounds elute (come out) first

HPLC High Preformance liquid chromatography

more sensitive

>Normal phase

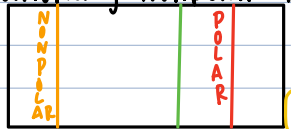
Stationary = polar mobile = nonpolar



- more effective!! faster!!
- nonpolar elutes first

>Reverse Phase *more common

Stationary = nonpolar mobile = polar

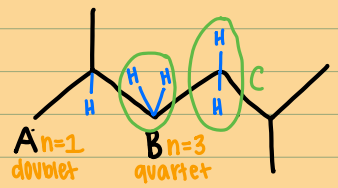


most polar elutes first

what's the benefit?

coat beads w/ hydrocarbons!

Question!!!
Splitting of A + B?



My answer:
A: doublet
B: Quartet

TPR answer:
A: doublet
B: doublet

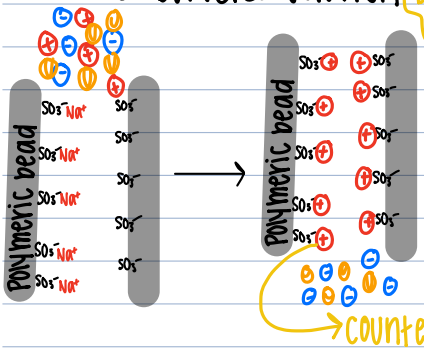
explaination:
MOLECULE IS SYMMETRICAL!!
B + C Hs are in the SAME chemical environment!!
same environment = doesn't split!!!

Ion Exchange Chromatography

charged bio molecules

difference in charged states
used when you can adjust charges
Stationary: resin containing anionic/cationic group w/ counter ion
Mobile: buffered solution — buffer keeps pH + charged states constant

counter ion = (+) = cationic exchange



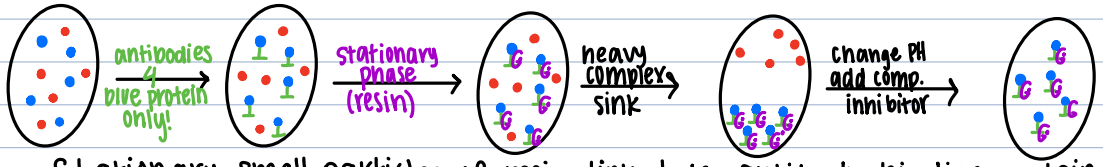
to get (+) out, you can change pH, or flush w/ concentrated soln.
Also works in reverse to isolate anions

counter ion = (+) then anionic stationary phase

anion resin → anions elute first. opp. for cation resin

Affinity Chromatography

highly specific lock + key interactions, enzymes, ligand, antibodies, etc.
used to separate proteins fm. blood or cell lysate



Stationary: small particles of resin-linked to antibody-binding protein
magnetic beads may serve as an alternative stationary phase
to elute target protein add competitive antibonding protein

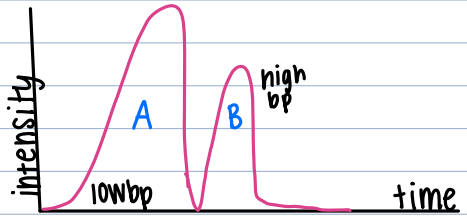
Gas chromatogy

separates by boiling point / volatility
small amounts of low bp compounds

used 4 information more than separation

Volatality: tendency of molecule to convert to gas
mobile: carrier gas, inert stationary: liquid absorbant, retains high bp compounds
low bp compounds exit first

> Output:
1. # of compounds = # of peaks
2. relative quantity = peak area
3. voltality / bp = time axis



lowest bp elute first

Distillation + Boiling Points

> Boiling Point

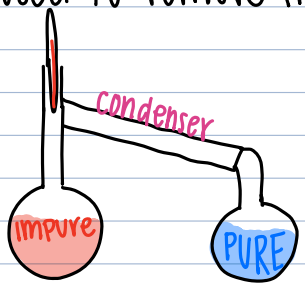
measure of intermolecular forces btwn liquids
- H bonds — Strongest
- Dipole-Dipole
- LDS — weakest

Other factors...
1. Molecular weight
   heavier = higher bp
2. Branching
   more branches = higher bp

> Simple Distillation

separate large amounts of compounds w/ low bp, but large diff. in their bp (>30°C)

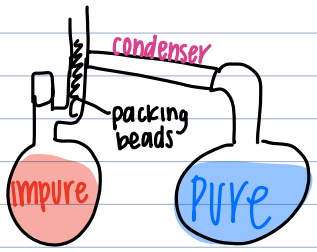
used to remove impurities



lower bp leaves first?

> Fractional Distillation

separate when bp of compounds is within 30°C
good for diastereomers



packing beads incr. surface area

lower bp leaves first?

Solvent Extractions

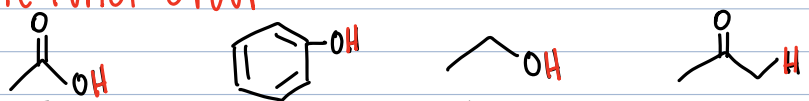
extractions separate compounds based on solubility differences

> Solubility

1. polar is soluble in polar ($\rightarrow H_2O$)
2. np is soluble in np

<5C w/ polar group = water soluble
charged functional groups are more soluble in water than organic

Acidic funct. Group



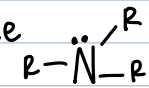
Pka: 5 — 10 — 15 — 20

10^5 x more acidic

★ phenols, carboxy acids + amines are main ones 4 extraction

Basic Funct. Group

amine



deprotonating acids/ protonating bases makes them charged + more H_2O soluble

> conditions

1. Aqueous $NaHCO_3$ (weak base): ONLY deprotonates carboxy. acid
   $RCOOH + NaHCO_3 \rightarrow RCOO^{\ominus}\ Na^{\oplus}$
2. Aqueous NaOH (strong base): deprotonate phenols AND carboxy. acid
   $Ph\text{-}OH + NaOH \rightarrow Ph\text{-}O^{\ominus} + Na^{\oplus}$
   ★ alkyl alcohols not acidic enough for this
3. Aqueous HCl (acid): protonates amines
   $R_3N + HCl \rightarrow R_3NH^{\oplus}\ Cl^{\ominus}$

Resolution of Enantiomers

only differ by optical activity

Resolution separates enantiomers of racemic mixture by...

1. converting enantiomers to diasteromeric salts w/ a chiral resolving agent (usu acid/base) must be enantiomerically pure
2. separate salts using normal methods (recrystallization)
3. Revert salts to original enantiomers, treat w/ base

Spectroscopy
color we see = reflected
complementary color = most absorbed

Mass Spectrometry
determine molecular weight
elemental + isotopic compensation

UV Vis. Spectroscopy
indicates presence of conjugated π system (sp^2 chain)
conj ↑ = more red
conj ↓ = more blue

IR Spectroscopy
IR light causes bonds to vibrate at distinct frequencies
indicates functional groups
used to monitor rxns
peaks are wavenumbers

good for distinct fxn groups

limitations
does not tell how many/where functional groups are
good for const. isomers, not stereoisomers

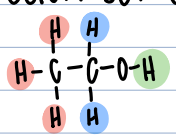
Group	Wave# (cm^{-1})
O-H	3200-3600 Broad
C=O	~1700 (variable)
C=C	1650
C≡N	2200-2500

C-H is never the answer! (2950)

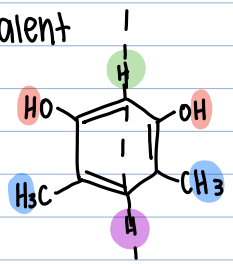
^{1}HNMR Spectroscopy

# of signals	# of nonequivalent H's
Splitting (n-1)	# of noneq. H neighbors
area under sig.	# H's rep. by signal
Chem. Shift	Chemical environment

> Noneq. Hydrogens
free rotation/symmetry makes H equivalent
each set of noneq. H have one peak

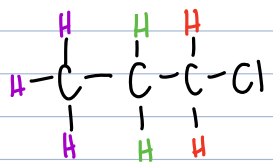


3 signals



4 signals

Splitting
indicates # of H on adj. carbons
n+1 = # peaks
must be w/in 3 σ bonds



H triplet
H triplet
H multiplet (6)

1 singlet
2 doublet
3 triplet
4 quartet
5+ multiplet

Integration
how many H represent each signal

(#H) (5H) (3H) etc.

Chemical shift
shifted due to ewdg/edg
downfield(←) desheilded, near ewdg
upfield (→) sheilded, near edg

aldehyde → aromatic → vinyl → N/O/X → alkyl
→ alcohol (2-5ppm)

After class
>FSQ
acetone 
most acidic compound shows greatest preference for NaOH (strong base)
(IR) ketone = 1715 cm^{-1} alcohol = 3500
cis vs trans (E vs Z) methyl gr. have protons w/ different chem. environments
TLC less polar = highest Rf
most basic compound shows higher affinity in acidic aqueous conditions
Distillation best for compounds w/ different sizes / imf (=bp).
in size exclusion chromatography components do (NOT) interact with the surface of the stationary phase
^{1}HNMR resonances of product = # of possible products
in radical bromination Br adds to LEAST substituted carbon!
☆☆ Don't forget about compound symmetry!! ☆☆☆
>passages
Distillation is used for liquids not solids
Methanol is imiscible w/ water