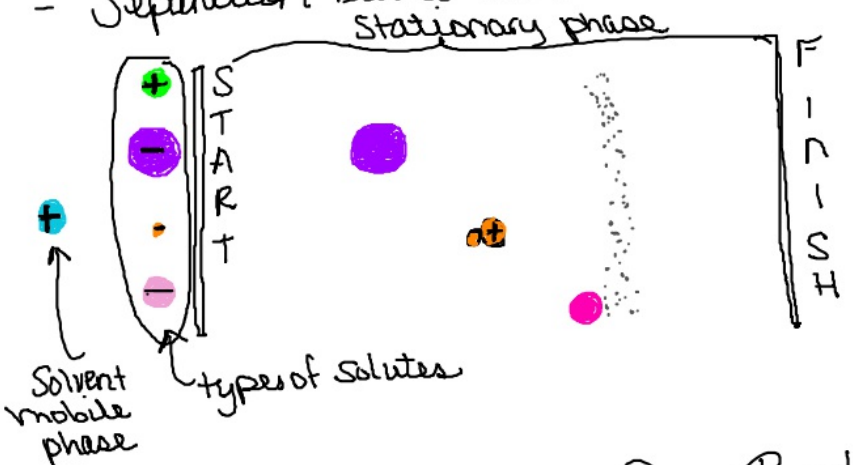
12/1/17 # Chromatography

- Solvents carry solutes through a medium leaving particles behind.
- Separation based on attraction + size



R_f value = distance travelled by substance / distance travelled by solvent

Signal for Chromatography is R_f = R_f value, R_f factor, Retention time

R_f = time spent in the stationary phase relative to the time spent in the mobile phase.

There are as many types of chromatography as there are combinations of mobile + stationary phases.

Mobile phase - solvent, carrier

Stationary phase - the media that will capture the substances or will adsorb or adhere to the substances.

What to consider when choosing the mobile & stationary phases —

1. Size of the particles
2. solubility of the solute in the solvent
3. Polarity or charge — is it a dipole? will it make hydrogen bonds? "like dissolves like"
4. Shape of the molecule
5. pH
6. phase of matter, boiling point
7. Temperature
8. Pressure
9. Selectiveness of stationary phase - some attraction but not so great that there isn't any movement.
10. mobile phase can not react w/ solute or stationary phase.

* Size → think about where the solute will "fall" out
  → Choosing void size (pore size) that allows some movement.

Type	Mobile Phase	Stationary Phase
Liquid Chromatography	liquid	adsorbent solid
Gas Chromatography	gas	packed bed of adsorbent material
Ion-Exchange Chrom.	liquid	resin with ions attached
Affinity Chromatography	liquid	Covalently bonded molecules on a solid surface
Partition Chromatography	liquid	thin film applied to a solid surface
Electrophoresis	electricity	agarose gel

Electrophoresis: based on the understanding that most macromolecules are charged ∴ they will move in an electric field. An electric current is used to carry particles through a stationary phase (agarose gel).

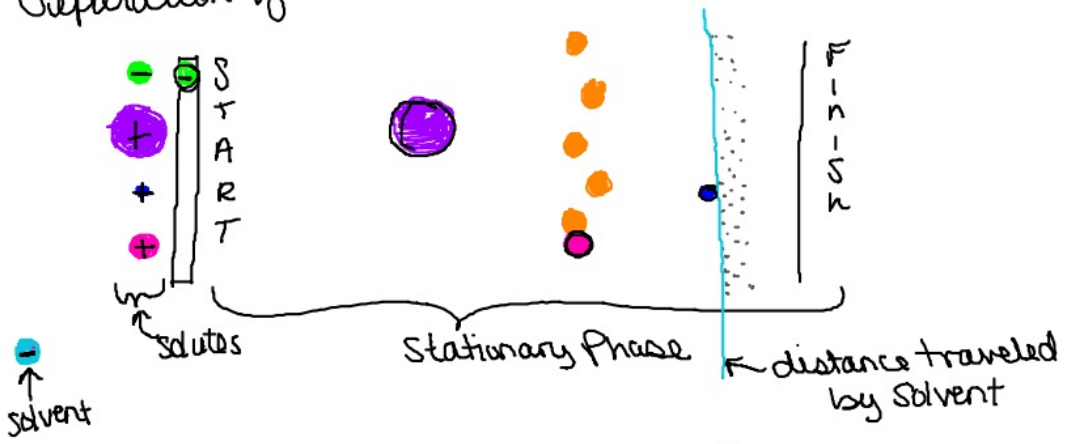
Most Common Errors: ① running it too long (removes separation)
② spilling over wells or tearing sample wells
③ handle agarose gel carefully – very soft
④ reversing electric field

Resolution – the distance between bands or particles

Chromatogram – the output (results) of the chromatography

12/1 Chromatography

- Solvents carry solutes through a medium leaving particles behind.
- Separation of a material based on attraction & size.



The signal for chromatography is the R_f value

Retention factor or Retention time

$$R_f = \frac{\text{distance solute travels}}{\text{distance solvent travels}}$$

R_f = the time spent in the stationary phase relative to the time spent in the mobile phase.

There are as many types of chromatography as there are combinations of mobile & stationary phases

Mobile phase – solvent, the carrier

Stationary Phase – the substance that will "capture" the solute
- by void (pore) size
- by adsorbing the solute
- by adhering to the solute

What to consider when choosing the mobile & stationary phases

1. phase of matter @ Room Temp.
2. Charge or polarity – ? dipole
   ? will it make hydrogen bonds
   remember "like dissolves like"
3. Void or Pore size, actual measurement of the stationary phase
4. Particle size & shape & mass
5. mobile phase viscosity & fluidity
6. Temperature
7. Pressure
8. Color – need to be able to see the chromatogram
9. pH
10. mobile phase & solute can not react
11. selectiveness of the stationary phase – some attraction but not so much that there is no movement or no differentiation of particles
12. Boiling Pt & Flash point
13. Solubility *

Main Types of Chromatography

Type	Mobile Phase	Stationary phase
Liquid Chrom.	liquid	adsorbent solid
Gas Chrom.	gas	packed bed of adsorbent material
Ion-Exchange Chrom.	liquid	resins with ions attached
Affinity Chrom.	liquid	covalently bonded molecules on a solid surface
Partition Chrom.	liquid	thin film applied to a solid surface
Electrophoresis	electricity	agarose gel

Electrophoresis: based on the understanding that most macromolecules are charged ∴ they will move in an electric field. An electrical current is used to carry particles through a stationary field. (agarose gel)

Most Common Errors: ① gel is very fragile
② over filling or puncturing the wells.
③ reversing the electric field

Resolution – distance between bands or particles, & visibility

Chromatogram – the output or results of the chromatography