Separations and Purifications

Extraction - transfer of a dissolved compound (desired product) from a starting solvent into a solvent in which the product is more soluble

- Based on the concept that **like dissolves like** - polar substances will associate polar solvents and nonpolar substances will dissolve in nonpolar solvents

When we perform extractions, the two solvents need to be **immiscible** - must form two layers that do not mix (like water and oil)

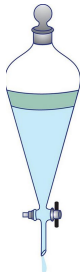
- The two layers are temporarily mixed by shaking so that solute can pass from one solvent to the other
- Example: in a solution of isobutyric acid and diethyl ether, if you add water and shake it up, the isobutyric acid will transfer over to the water layer because it is more soluble in a polar solvent like water
- **Aqueous layer** and **organic layer** make up the extraction

To then separate out the two layers, we use a **separatory funnel**, which uses gravitational forces to cause the denser layer to sink to the bottom of the funnel where it can be removed by turning the stopcock at the bottom

- It is **more common for the organic layer to be on the top** - not always the case (determined by density)

Extraction is entirely determined by solubility rules and that like dissolves like:

1. Hydrogen bonding - compounds that can do this, such as alcohols or acids, will move most easily into the aqueous layer
2. Dipole-dipole interactions - these compounds are less likely to move into the aqueous layer
3. Van der Waals - with only these, compounds are least likely to move into the aqueous layer



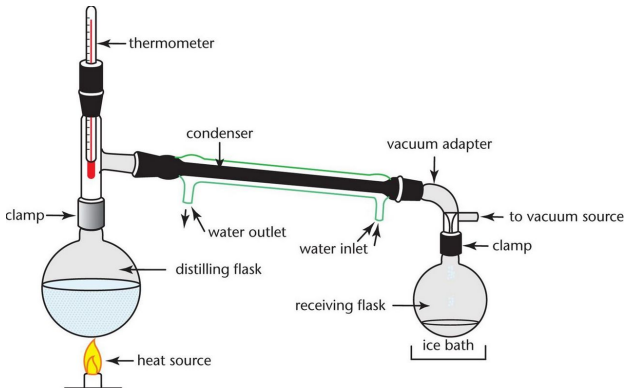
Distillation takes advantage of differences in boiling point to separate two liquids by evaporation and condensation

- The lower boiling point liquid vaporizes first, vapors rise up the distillation column to condense in a water-cooled condenser

- The **condensate** then drips down into a vessel and the end product is called the **distillate**
- The heating temperature is kept low so the liquid with the higher boiling point will not be able to boil and therefore will remain liquid in the container
- Often times a boiling or chip or **magnetic stirrer** will be introduced to break surface tension and prevent superheating - liquid is heated to a temperature above boiling point but does not vaporize

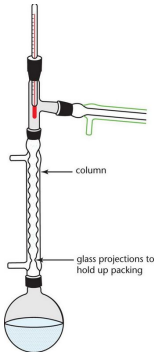
Vacuum distillation - used whenever we want to distill a liquid with a **boiling point over 150 Celsius**

- Using a vacuum lowers the ambient pressure, thereby decreasing the temperature that the liquid must reach to have sufficient vapor pressure to boil



Fractional distillation - used to separate liquids with **similar boiling points**

- A **fractionalization column** connects the distillation flask to the condenser - fractionalization columns have increased surface area by the inclusion of inert objects like glass beads or steel wool
- As the vapor rises up the column, it condenses on these surfaces and refluxes back down until rising heart causes it to evaporate again, only to condense again higher in the column
- Each time the condensate evaporates, the vapor consists of a higher proportion of the compound with the lower boiling point



Chromatography - operates under the basic principle that the more similar a compound is to its surroundings, whether by polarity, charge, etc.), the more it will stick to and move slowly through its surroundings

- Place a sample onto a solid medium called the **stationary phase** or **adsorbent**
- Then run the **mobile phase** (usually a liquid or a gas in gas chromatography) through the stationary phase
- This will displace (**elute**) the sample and carry it through the stationary phase and depending on the strengths/characteristics of the substances, it will adhere to the stationary phase with different strengths, causing different substances to migrate at different speeds - called **partitioning**

The MCAT most commonly tests chromatography that functions based on **polarity**

Thin layer chromatography - Very similar to **paper chromatography** and only varies in the medium used for the stationary phase - TLC uses a thin layer of **silica gel** and paper chromatography uses **cellulose composed paper**

- Sample is **spotted** on the stationary phase and the plate becomes **developed**, which involves placing the adsorbent upright in a developing chamber, usually a beaker with a lid or wide-mouthed jar
- At the bottom is a pool of solvent, called the **eluent** and the spots of the sample must be above the level of the solvent
- Then the sample will move up the plate by capillary action

TLC gel is **polar and hydrophobic** and the **organic solvent is weak to moderate polarity**, so it doesn't bind well to the gel

- Nonpolar compounds, thus, dissolve in the organic solvent and move quickly as the solvent moves up the plate, whereas more polar molecules stick to the gel - **the more nonpolar a sample is, the further up the plate it will move**

Reverse plate chromatography - The exact opposite, the stationary phase used is nonpolar, so polar molecules move up the plate quickly and nonpolar molecules stick more tightly to the stationary phase

Most spots are white so developed plates are placed under **UV light**, which will show any compounds that are ultraviolet sensitive

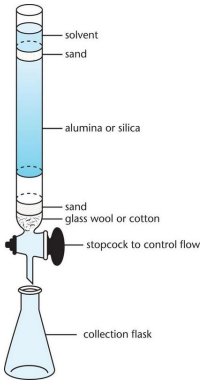
Compounds are identified using the **retardation factor (R_f)**, which is relatively constant for a particular compound in a given solvent

R_f = distance spot moved / distance solvent front moved

Column chromatography - Uses an entire column filled with silica or aluminum beads as an adsorbent/stationary phase, allowing for much greater separation

- Uses **gravity** to move the solvent and compounds *down* the column

- The more similar the sample is to the mobile phase, the faster it elutes; the more similar to the stationary phase, the more slowly it will elute (if at all)
- Useful in biochemistry because it is used to separate and collect macromolecules such as proteins and nucleic acids



- **Ion exchange chromatography** - beads in the column are coated and charged so they attract or bind to compounds that have the opposite charge
    - After completion, a **salt gradient** is used to elute the charged molecules that have stuck to the column
- **Size exclusion chromatography** - beads in the column contain tiny pores of varying sizes that allow **small compounds** to enter and slow them down, large can't fit and travel faster
- **Affinity chromatography** - protein of interest is bound by creating a column with high affinity for that protein (coating beads with a receptor that binds that protein or a protein antibody), which causes the protein to be retained in the column

Gas chromatography (GC) - Also known as vapor-phase chromatography - **eluent is a gas** (hydrogen or helium) instead of a liquid

- The adsorbent is a crushed metal or polymer inside a 30-foot column that is **coiled** and kept inside an oven to control its temperature
- The mixture is then injected into the column and vaporized and the gaseous compounds will travel through the column at different rates because they adhere to the adsorbent in the column to different degrees and will separate in space by the time they reach the end of the column
- The injected column must be **volatile** - low melting point, sublimable solids, or vaporizable liquids
- The compounds then get registered by a **detector**, which records them as a peak on a chart

High performance liquid chromatography - the eluent is a liquid and it travels through a column of a defined composition

- Small sample is injected into the column and separation occurs as it flows through

- The compounds pass thru a detector and are collected as the solvent flows out of the end of the apparatus
- Very similar to GC, but uses liquid instead