Ion Exchange Chromatography

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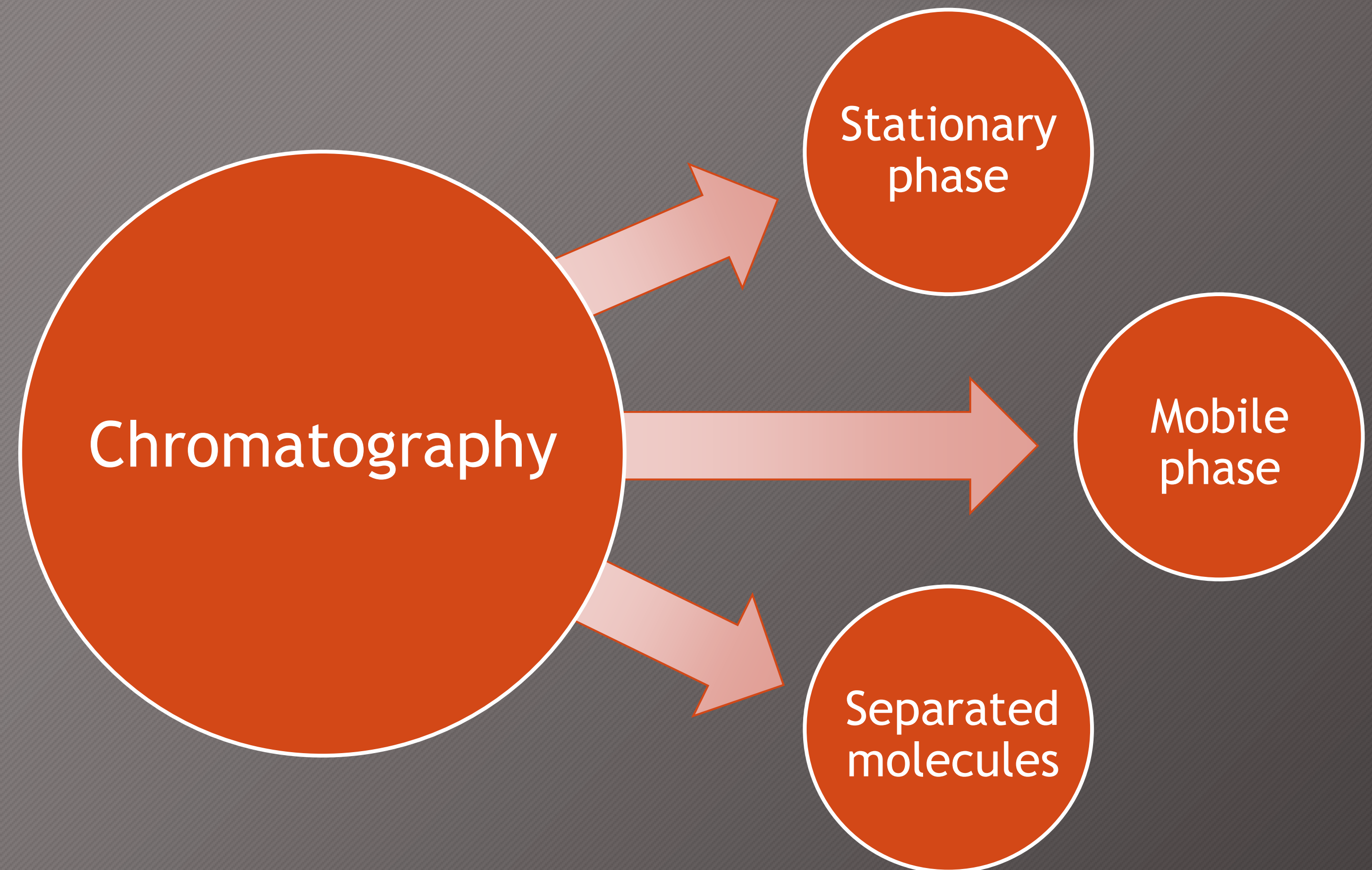
March 4, 2019

Chromatography

- Is a technique used in the separation and purification of the components of a mixture for qualitative or quantitative analysis.
- Separation occurs due to the interaction between the components and depends on factors such as: affinity, adsorption, partition or molecular weight.
- Some types of chromatography:
  - Paper chromatography
  - Ion exchange chromatography
  - Gas chromatography
  - HPLC
  - TLC



Ion Exchange Chromatography

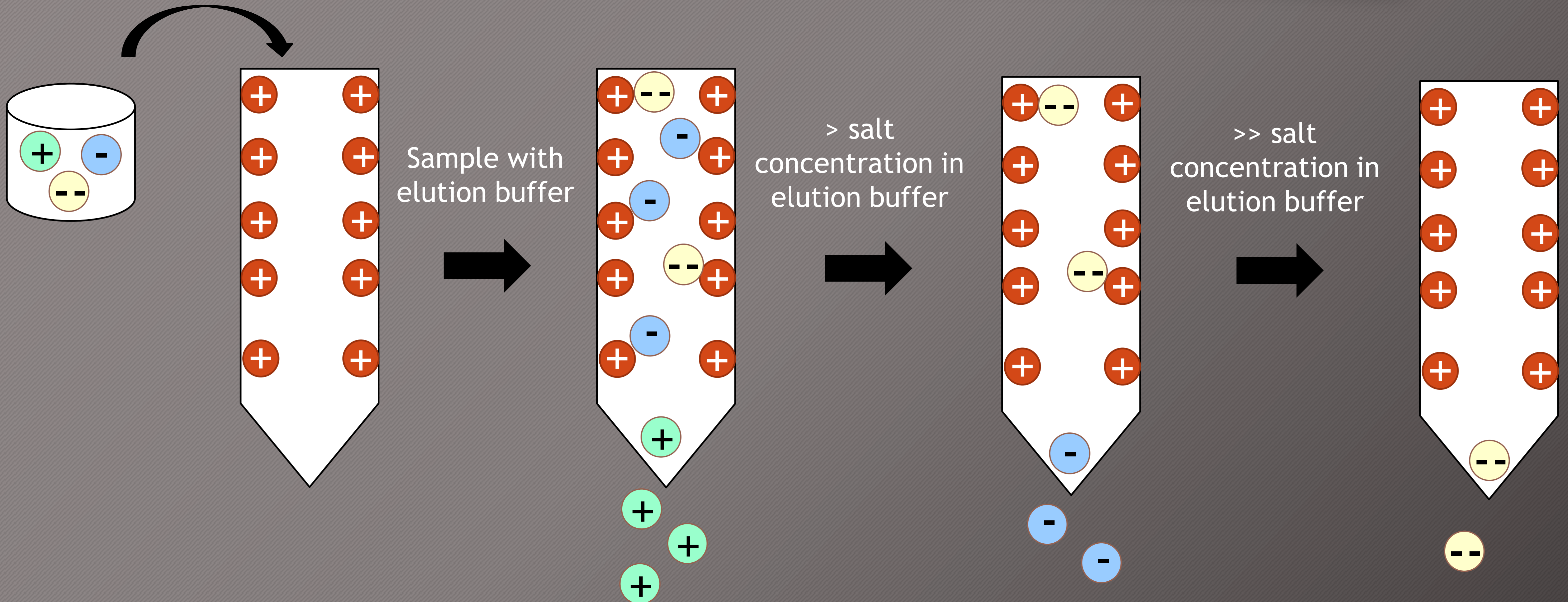
What is IEC?

- Is a technique used to separate ionizable molecules based on their total charge.
  - Commonly used to separate amino acids, proteins and nucleotides from complex biological samples
- Can be used for preparative or analytical purposes.

How does it work?

1. The resin is composed of a positively or negatively charged functional group bound to a solid matrix.
2. Oppositely charged molecules of the sample will bind to the resin with different strengths.
3. Selective release of these adsorbed molecules occurs by:
   - increasing the ionic strength of the elution buffer = salt ions will compete for the resin functional groups
   - changing the pH of the elution buffer = when the pH reaches the isoelectric point of a protein (No net charge) it will elute from the column.

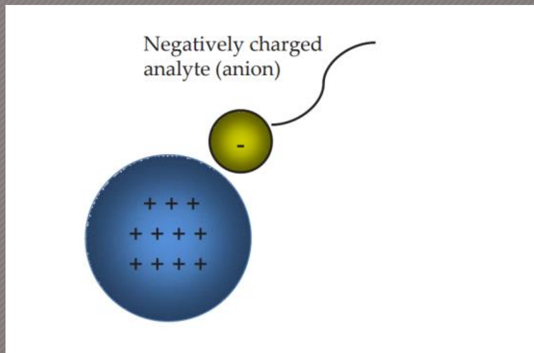
Ion Exchange Chromatography



Types of Ion Exchange Chromatography

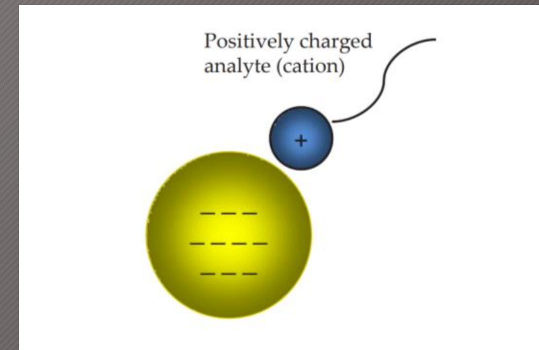
Anion Exchange Chromatography

- Uses a positively charged ion exchange resin that will bind negative molecules.
- “Anion exchanges anion”

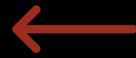


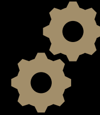
Cation Exchange Chromatography

- Uses a negatively charged ion exchange resin that will bind positive molecules.
- “cation exchanges cation”

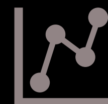


General Protocol


Select resin


Buffer preparation

Run sample through column


Data Analysis

Factors to consider in resin selection

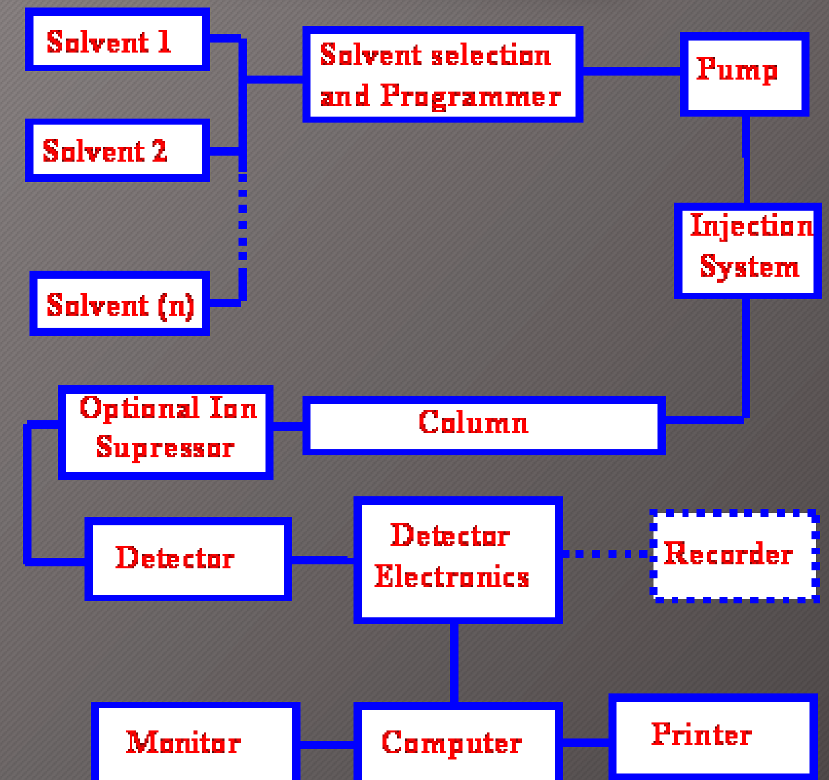
- Resin can be positively or negatively charged.
  - Will depend on protein stability.
- Resin can be weak or strong.
  - Strong: Number of charges on resin stays constant regardless of buffer pH; they retain their selectivity and capacity over a wide range of pH.
  - Weak: their functionality is pH dependent; have a small range of pH where they provide optimal performance.
- Resin particle size.
  - Smaller size: Higher resolution but requires slower flow rates; not effective for viscous samples.
  - Larger size: Higher flow rate but lower resolution; convenient for large scale/ preparative work and viscous samples.
- Dynamic Binding Capacity of Resin.
  - Reported as mg/ml of protein bound at certain flow rate; Important when fast flow rates are required to maintain protein activity.

Buffer Preparation

- Prepare a loading, wash and elution buffer.
  - If you want elution to depend on ionic strength: have a buffer with no/low salt (buffer A) and one with high salt (buffer B).
- Composition is of great importance!
  - The counterion in the buffers should be compatible with the resin and serve its purpose.
  - 10-25mM is fine.
  - Sometimes reducing agents are needed to maintain protein native, such as DTT. (prepare it fresh for purification with 0.1-1mM)
- Readjust pH after adjusting salt concentration.
- Buffer of sample should be the same as the starting buffer.
- Degassing and filtering of buffer before experiment is necessary.

IEC Steps

1. Column washing with high salt buffer
2. Equilibrate column
3. Sample loading
4. Column washing
5. Elution
6. Column stripping and equilibration



General instrumentation for IEC

IEC Steps

1. Wash column with high salt buffer.
   - To eliminate any protein residue from previous use.
   - If column was in ethanol, pass water through it for 20min at 1m/min to have an aqueous background.

2. Equilibration of column
   - Equilibrate column until pH and conductivity readings stabilize with loading buffer.
   - Requires 5-10 column volumes of buffer.

3. Sample loading.
   - When possible, samples should be in starting buffer.
   - For a sample loop of 500μL max volume, use 100-500μL of sample.

4. Column washing.
   - Wash the column in loading buffer (0% buffer B) until no protein is detected in the flowthrough.
   - Typically requires 3-5 column volumes.

IEC Steps

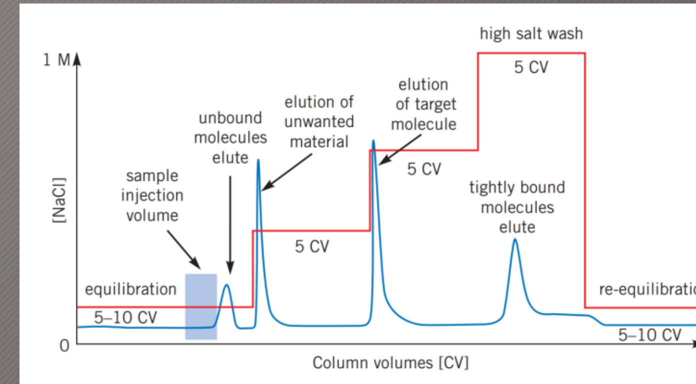
4. Elution

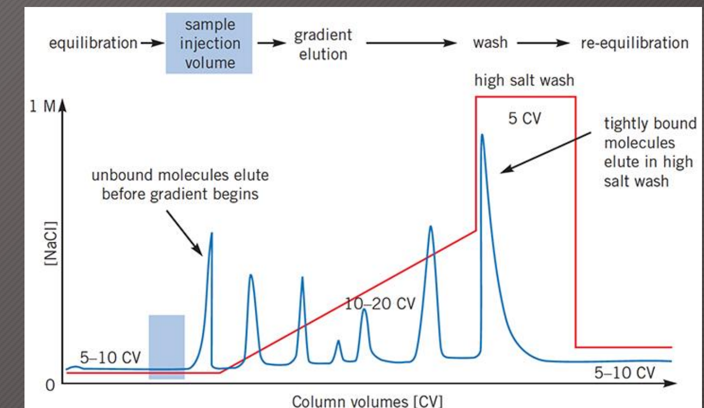
Step isocratic elution

- Uses increasingly stronger isocratic steps over time.
- Convenient when the separation has been optimized.
- Provides high resolution and maximum purification.
- Reduces buffer consumption and purification time.

Gradient elution

- Gradually increases strong solvent percent over time.
- Convenient to optimize elution conditions.
- Simple purification process that may give good resolution.
- Use a 5-10 column volume gradient as a starting point.





IEC Steps

4. Elution: Flow rate
   - Determines how fast a buffer passes over the resin
   - Lower flow rate, higher resolution and capacity.
   - Is limited by the resin being used.
   - Use the fastest flow rate that will still yield the resolution and capacity desired.
   - Recommendation: 1-2mL/min for equilibration and washing; 0.5mL/min for elution.

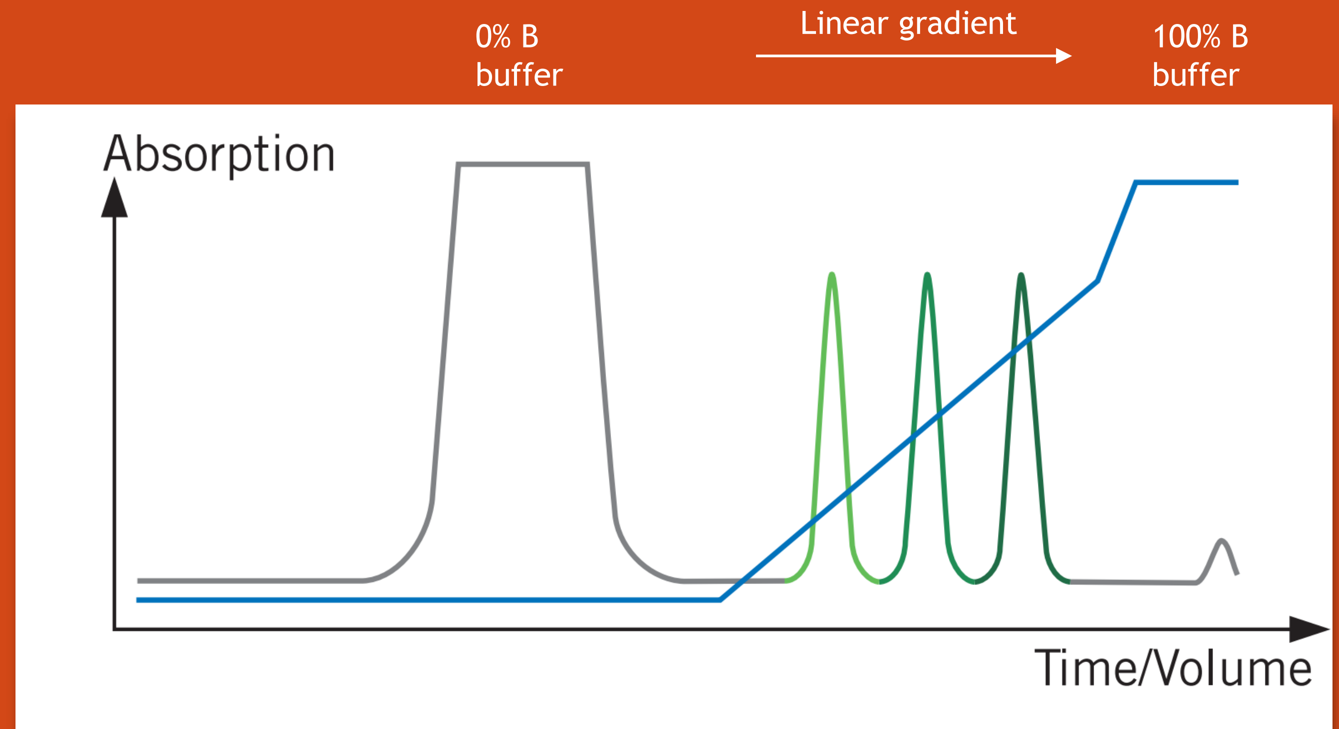
5. Column stripping and equilibration.
   - After obtaining protein of interest, eliminate the rest of the proteins by using a high salt buffer (100% buffer B; Ex. 1M NaCl).
   - Equilibrate with starting buffer.
   - Note: A way to prevent microbial growth is to use 20% ethanol in water for long-term storage of column.

Data Analysis

- UV-Vis and conductivity will show the elution peaks and changes in salt concentration as a function of time or volume.
    - Protein absorbance peaks: 280nm (aromatic groups) and 220nm (backbone)

- Collect the fractions of interest and perform analytical studies, such as Bradford assay (total protein content), SDS-PAGE (purity determination), etc.

- After determining which fractions have the protein of interest, you can do with it as you please.
    - Dialyze
    - Concentrate with ammonium sulfate precipitation
    - Desalt
    - Store for later analysis.



For anion exchange chromatography, first elution peak corresponds to less negatively charged species and last peak to the most negatively charged species.

Considerations

- Fraction collection volume can be from 1/5th to 1/10th of the column volume.
- Temperature must remain constant through the run because it may affect the conductivity measurements.
- If precipitate is present, filtrate or centrifuge samples before injection to avoid damaging the column.

Thank you for your attention!