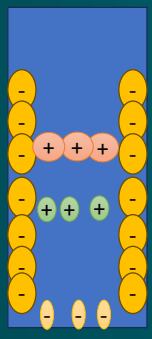
Ion Exchange Chromatography

(Part 1)



Chromatography
Instrumental Analysis

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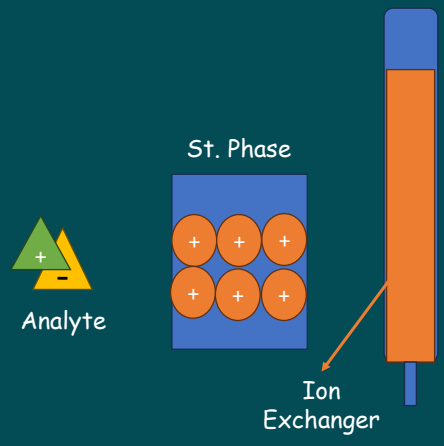
Ion Exchange Chromatography

Introduction & Principle

- Ion Exchange Chromatography or Ion Chromatography is the techniques of separation of ions (Charged particles) and polar based on their affinity to ion exchangers.
- It is mainly used to separate proteins, peptide, amino acid, and Nucleic acid.
- The basic principle is reversible exchange of ions and attraction between opposite charged



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Ion Exchange Chromatography

Ion Exchanger

- **Cation Exchangers**/ Acidic ion exchanger- Attract Cation
- $-COO^-$, $-CH2-COO^-$, SO_3^- , $-CH2-SO_3^-$
- Negative charged buffer like Acetate, phosphate, sulphate, lactate, succinate can be used with cation exchanger

- **Anion Exchangers**/ Basic ion exchanger- Attract Anion
- $CH_3CH_2NH_3^+$, $-CH_2N^+(CH_3)_3$
- Positive charged buffer like pyridine, Tris buffer can be used

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Ion Exchange Chromatography

Ion Exchanger

Increase 1 unit pH- Net (-)ve Charged → **Anion Exchanger**

Protein

At isoelectric pH- Net Zero Charged

Decrease 1 unit pH- Net (+)ve Charged → **Cation Exchanger**

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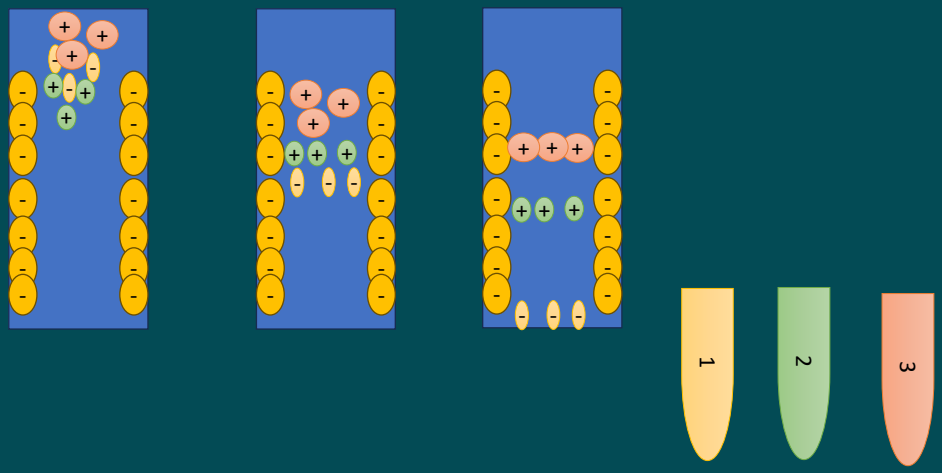
Ion Exchange Chromatography

Cation Exchanger



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Ion Exchange Chromatography

Pros and Cons

Pros-

- ✓ High flow rate
- ✓ Concentrate the sample
- ✓ High Yield
- ✓ Buffer do not denaturants

Cons-

- ✓ Sample must be loaded at low ionic strength
- ✓ Small changes in pH can affect the results
- ✓ Particle size influence the resolution

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Ion Exchange Chromatography

(Part 2)

Ion Exchange Resins
Applications

Chromatography
Instrumental Analysis

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Ion Exchange Chromatography

Ion Exchange Resins

According to chemical Nature they are classified as

A. Strong Cation Exchange Resin- -SO3H SO_3H
B. Weak Cation Exchange Resin- -COOH, -OH, -SH, PO3H2 PO_3H_2
C. Strong Anion Exchange Resin- N^+R3, -NR2 NR_2
D. Weak Anion Exchange Resin- -NHR, -NH2 NH_2

According to Sources they are classified as

A. Natural: 1. Cation- Zeolytes, Clay
   A. 2. Anion- Dolomite
B. Synthetic- Inorganic and Organic Resins

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Ion Exchange Chromatography

Class of resin	Nature	pH range	Applications
Cation - Strong	Sulfonated polystyrene	1 - 14	-fractionation of cations -inorganic separations (lanthanides) -peptides, amino acids, B vitamins
Cation - Weak	Carboxylic methacrylate	5 - 14	-fractination of cations -biochemical separations -organic bases, antibiotics
Anion - Strong	Quaternary ammonium polystyrene	0 - 12	-fractionation of anions -alkaloids, vitamins -fatty acids
Anion - Weak	Polyamine polystyrene or Phenol formaldehyde	0 - 9	-fractionation of anionic complexes -anions of different valency -vitamins, amino acids

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Ion Exchange Chromatography

Ion Exchange Resins

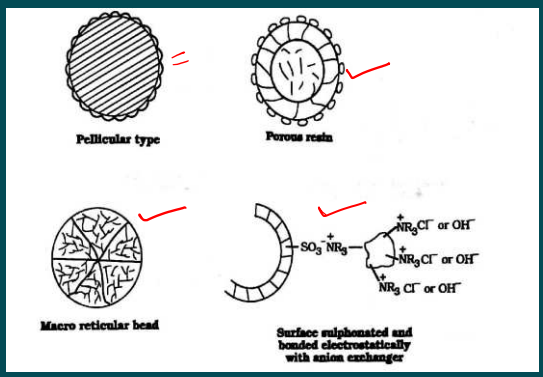
According to structural Nature they are

A. **Pellicular Type-** Size 30-40 um with 1-2um film thickness, very low exchange capacity (efficiency- 0.01-0.1 meq/g of ion exchange resin)

B. **Porous Resin Coated with exchanger beads-** Size- 5-10 um, high efficient. Exchange capacity- 0.5-2 meq/g of ion exchange resin)

C. Macroreticular resin bead-

D. Surface Sulfonated and bonded electrostatically with anion exchanger. Less efficient (0.02 meq/g of exchange resin)



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Ion Exchange Chromatography

Ion Exchange Resins

- Mostly Organic ion resins are widely used, and these are polymeric resin matrix containing exchange site.
- The resin composed of Polystyrene and Divinyl Benzene
  - Polystyrene contains site for exchangeable functional group
  - Divinylbenzene act as a cross linking agent

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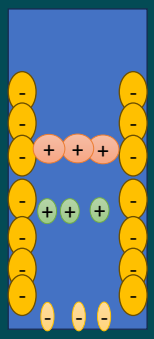
Ion Exchange Chromatography

Practically Requirement

- 1. **Column**:
  - Laboratory column (made-up of Glass), industrial column (stainless steel or polymer which resist to acid and alkali)
  - Length:diameter- 20:1 to 100:1
- 2. **Ion Exchange Resin beads**
- 3. **Mobile Phase**: Buffers (phosphate, acetate, borate, phthalate buffers), 0.1 N HCL, 0.1N NaOH



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Ion Exchange Chromatography

Factors Affecting Ion Exchange Seperation

- 1. **Nature of ion exchange resin**:
  - Crosslinking and swelling is important factors
  - More Crosslinking- more rigid but swell less, so separation of ion of different size is difficult, and become selective for specific size
  - Less crosslinking- less rigid, swell more, so separation will not be efficient due to wide pore.
- 2. **Nature of Exchanging ions**
  - Valency: $Na^+ < Ca^{2+} < Al^{3+} < Th^{4+}$
  - Size of ion: increase with decrease in size if same charge particle available.
    - $Li^+ < H^+ < Na^+ < K^+ < Rb^+ < Cs^+$
  - Polarizability : exchange is preferred for greater polarizable ion. $I^- < Br^- < Cl^- < F^-$

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Ion Exchange Chromatography

Factors Affecting Ion Exchange Separation

- 2. **Nature of Exchanging ions**
  - Concentration: in dilute solution, polyvalent anions are generally absorbed preferentially.
  - Conc. and Charge of ion:
    - If resin has higher +ve charge and solution has lower +ve charge, exchange is favoured at higher concentration.
    - If the resin has lower +ve charge and solution has high +ve charge. then exchange is favoured at low concentration.

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Ion Exchange Chromatography

Application:

- Softening of water: by removing ions
- Demineralization of water
- Purification pf some solution'
- Separation of inorganic ions
- Separation of proteins, amino acids, nucleic acid
- Biochemical separation'
- Concentration of ionic solution
- Ion exchange column in HPLC

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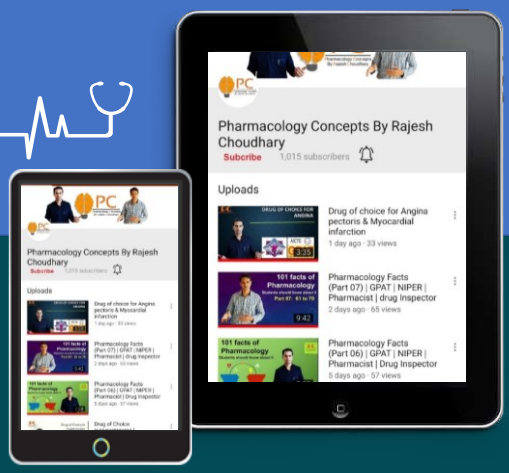
Thanks for Watching



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