Ion Exchange Chromatography

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Prerequisites

- Knowledge of relative polarity
- Concept of Hydrophobicity and Hydrophilicity
- Concepts of Liquid Chromatography

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Ion Exchange Chromatography

- ✓Ion exchange chromatography -- is a separation based on charge
- ✓Used for almost any kind of charged molecules --- large proteins, small nucleotides and amino acids
- ✓Ion-exchange chromatography preserves analyte molecules on the column based on ionic interactions
- ✓Mobile phage – buffer, pH and salt concentration--- opposite charged solute ions attracted to the stationary phage by electrostatic force
- ✓Stationary phage– resin is used to covalently attach anions or cations onto it

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Principle…….

- Ion Exchange Chromatography relies on charge-charge interactions between the proteins

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Types of IEC….

- anion exchangers
- cation exchangers

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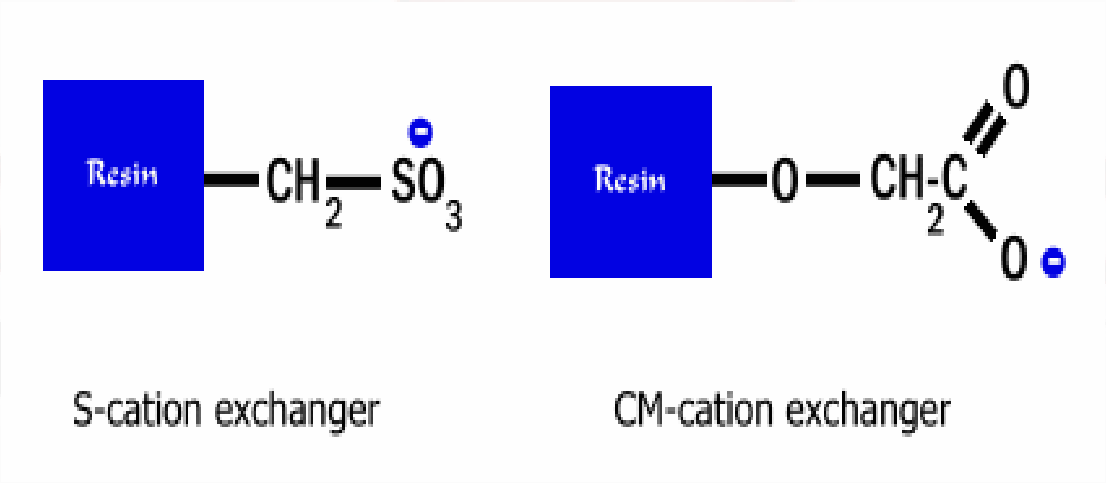
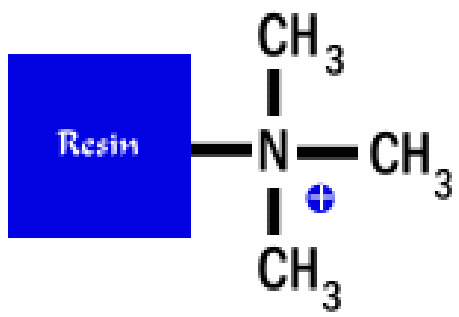
Cation exchange chromatography

Positively charged molecules are attracted to a negatively charged solid support. Commonly used cation exchange resins are S-resin, sulfate derivatives; and CM resins, carboxylate derived ions

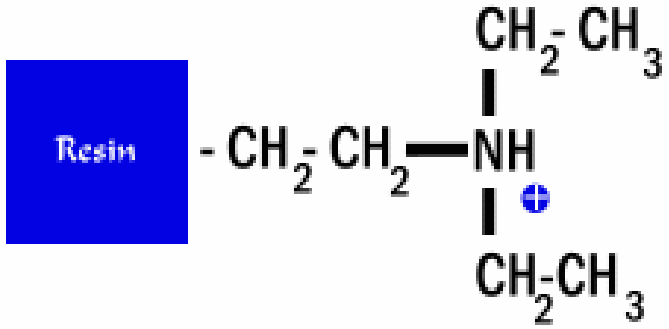


Anion exchange chromatography

Negatively charged molecules is attracted to a positively charged solid support. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, DiEthylAminoEthane



Q-anion exchanger



DEAE-anion exchanger

Buffers Used In IEC

✓Buffer system 1 : Buffer A = 20 mM Tris, pH=8. Buffer B = 20 mM Tris, 1 M NaCl, pH=8.0

✓Buffer system 2: (Common CEC buffer system): Buffer A = 30 mM sodium acetate, pH=4.5

Buffer B = 30 mM sodium acetate, 1 M NaCl, pH=4

✓Buffer system 3: (AEC for proteins which are very insoluble or have a very high pI)

Buffer A = 30 mM Ethanolamine, 8M urea, pH=10.0

Buffer B = 30 mM Ethanolamine, 8M urea, 1 M NaCl, pH=10.0

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Chromatography Methods

- ✓Column washed with buffer A to equilibrate
- ✓Buffer B is used to equilibrate again
- ✓Equilibrate the column with buffer A
- ✓Sample loading
- ✓Flow through collection
- ✓Elute protein

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Advantages

✓It is a non-denaturing technique. It can be used at all stages and scales of purification

✓An IEX separation can be controlled by changing pH, salt concentration and/or the ion exchange media

✓It can serve as a concentrating step. A large volume of dilute sample can be applied to a media, and the adsorbed protein subsequently eluted in a smaller volume

✓It offers high selectivity; it can resolve molecules with small differences in charge.

Disadvantages

- ✓costly equipment and more expensive chemicals
- ✓turbidity should be below 10ppm.

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References:

1. *Principles of Ion Exchange Chromatography". separations.us.tosohbioscience.com. Retrieved 1 May 2018.*

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3. Dąbrowski, A., Hubicki, Z., Podkościelny, P., & Robens, E. (2004). Selective removal of the heavy metal ions from waters and industrial wastewaters by ion-exchange method. Chemosphere, 56(2), 91-106.