BHARATHIDASAN UNIVERSITY

Tiruchirappalli – 620024,
Tamil Nadu, India.

Programme: M.Sc., Botany

Course Title : CELL BIOLOGY AND BIOINSTRUMENTATION
Course Code : 22PGBOT104

Unit – V

SEPARATION TECHNIQUES

Topic: Ion Exchange Chromatography

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ION EXCHANGE CHROMATOGRAPHY

Chromatography

- The process or technique of separating molecules or components in a mixture solution (gas or liquid) according to the differential absorption and elution
- Invented in 1906 by the Russian botanist Mikhail Tsvet
- Chromatography is the physical separation of a mixture into its individual components.
- Used in qualitative and quantitative analysis of biological and chemical substances

- This technique employs two immiscible substances- mobile phage and stationary phage

- Mobile phage

  -solution of gas or liquid components, works as transporter, moves in a definite direction

- Stationary phage

  -liquid or solid, absorbs or impedes different components of the solution to different degrees

Principle……

Reversible exchange of ions between ions present in the mobile phase and ion exchange resin.

Types of Chromatography

- ✓Adsorption Chromatography
- ✓Partition Chromatography
- ✓Ion Exchange Chromatography
- ✓Molecular Exclusion Chromatography
- ✓Affinity Chromatography

Ion Exchange Chromatography

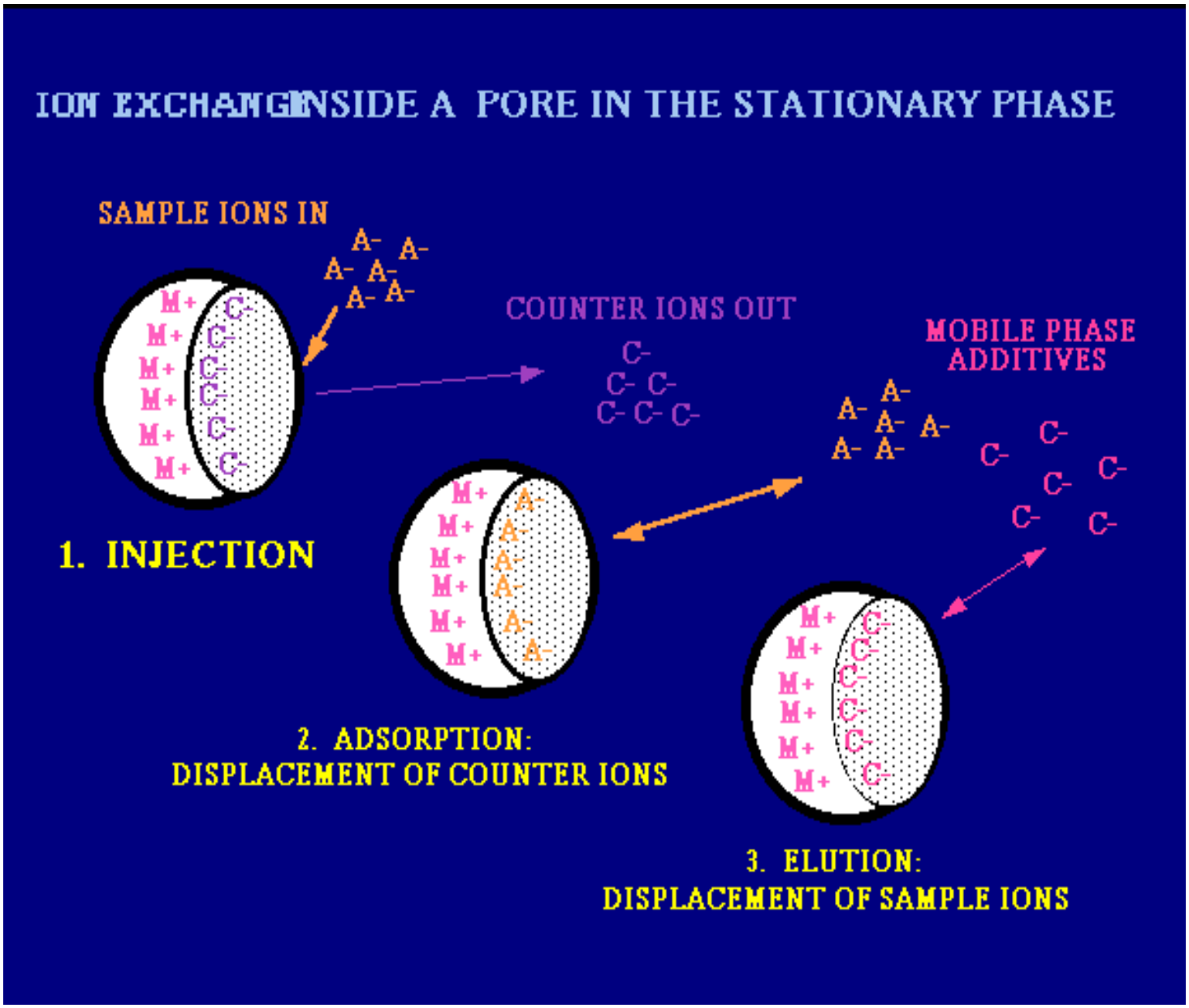
Ion exchange chromatography -- is a separation based on charge

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 phase – buffer, pH and salt concentration---opposite charged solute ions attracted to the stationary phage by electrostatic force

Stationary phase– resin is used to covalently attach anions or cations onto it


ION EXCHANGE INSIDE A PORE IN THE STATIONARY PHASE
SAMPLE IONS IN
A-
COUNTER IONS OUT
C-
MOBILE PHASE ADDITIVES
M+
1. INJECTION
2. ADSORPTION:
DISPLACEMENT OF COUNTER IONS
3. ELUTION:
DISPLACEMENT OF SAMPLE IONS

Principle…….

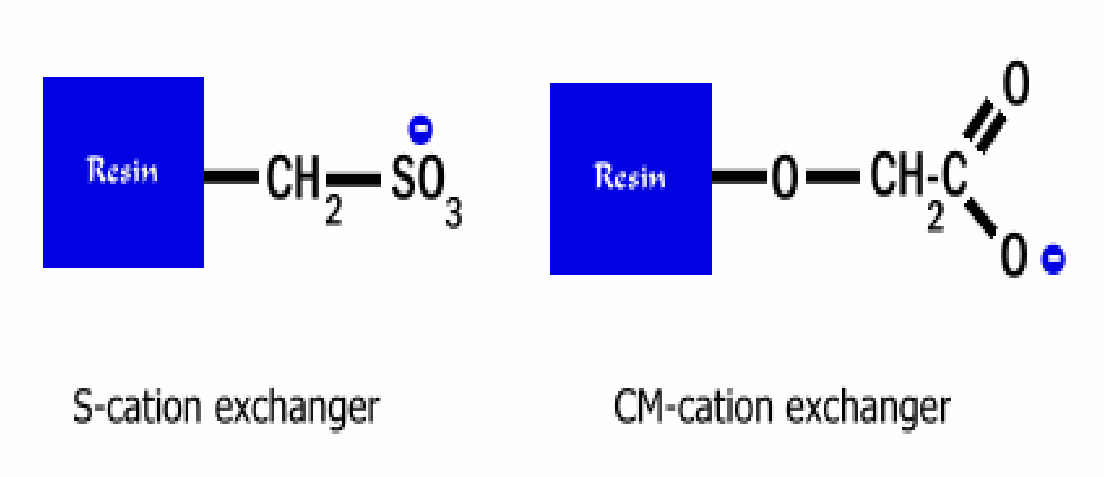
- Ion Exchange Chromatography relies on charge-charge interactions between the proteins

Types of IEC….

- anion exchangers
- cation exchangers

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

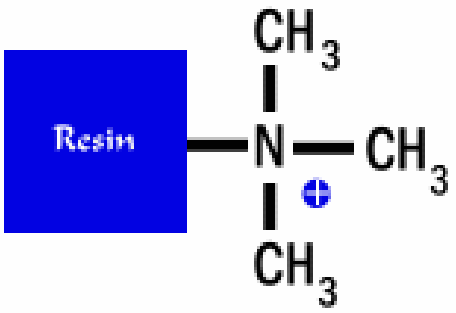


S-cation exchanger

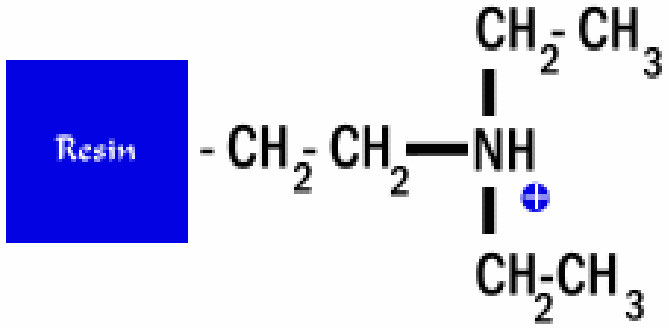
CM-cation exchanger

Anion exchange chromatography

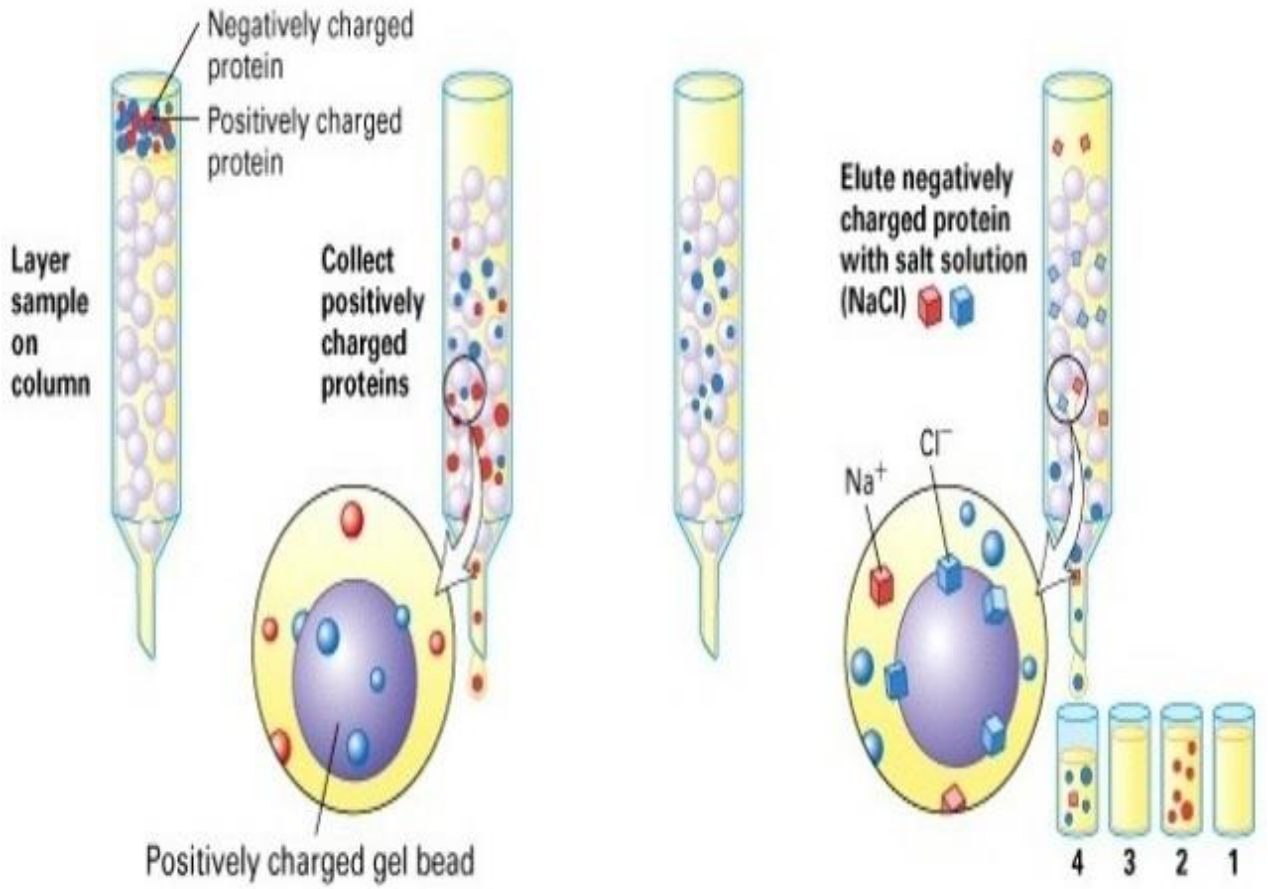
---negatively charged molecules is attracted to a positively charged solid support. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, DiEthylAminoEthane



Q-anion exchanger



DEAE-anion exchanger


Negatively charged protein
Positively charged protein
Layer sample on column
Collect positively charged proteins
Positively charged gel bead
Elute negatively charged protein with salt solution (NaCl)
Na+
Cl−
4
3
2
1

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

Recommended Buffers for Polypeptide Ion-Exchange Chromatography

A wide range of buffers are available for use with ion-exchange chromatography. Recommended buffers for various ranges of pH are listed below.

Anion-Exchange Chromatography Buffers

Buffers for anion exchange are generally basic amines.

Buffer	Concentration	Anion	pKa	Buffering Region
L-histidine	20 mM	Cl-	6.15	5.5 - 6.8
bis-Tris	20 mM	Cl-	6.50	5.8 - 7.0
bis-Tris propane	20 mM	Cl-	6.80	6.4 - 7.3
Triethanolamine	20 mM	Cl-	7.77	7.3 - 8.2
Tris	20 mM	Cl-	8.16	7.5 - 8.8
diethanolamine	20 mM	Cl-	8.88	8.4 - 9.4

Cation Exchange Chromatography Buffers

Buffers for cation-exchange chromatography are acids.

Buffer	Concentration	Cation	pKa	Buffering Region
formate	20 mM	Na+	3.75	3.3 - 4.3
acetate	20 mM	Na+	4.76	4.2 - 5.2
MES	20 mM	Na+	6.15	5.5 - 6.7
phosphate	20 mM	Na+	2.1/7.2	2.0 - 7.6
HEPES	20 mM	Na+	7.55	7.6 - 8.2

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

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.

Conclusion

Ion exchange chromatography is more efficient than other chromatography. It could be widely used for commercial purposes.

Affinity chromatography

- based on a highly specific biologic interaction suc h as that between antigen and antibody,
  enzyme and substrate, or receptor and ligand.
  Any of these substances, covalently linked to an insoluble support or immobilized in a gel, may

  serve as the sorbent allowing the interacting subs tance to be isolated from relatively impure sampl es; often a 1000-
  fold purification can be achieved in one step.

- This form of chromatography is growing in use. Lectins are glycoproteins have an affinity for carbohydrate residues. For example, they can separate polysaccharides, glycopeptides, and oligosaccharides and cells that contain particular carbohydrate structures.

THANKS