Ion Exchange Chromatography

Ion exchange chromatography is applicable for the separation of charged molecules. In this chromatographic technique, ionic solutes display reversible electrostatic interactions with a charged stationary phase. The stationary solid phase commonly consists of an insoluble matrix with covalently attached anions or cations (called ion exchangers). Solutes entering the column may be -vely charged, +vely charged, or neutral under the experimental conditions.

Solute ions of the opposite charge in the mobile liquid phase bind reversibly to the ion exchanger by electrostatic interactions. The strength of interactions depends on the size of the charge and the charge density (amount of charge/unit volume) of the solute. The greater the charge or charge density, the stronger the interaction. Neutral solutes show little or no affinity for the stationary phase and move with the eluting buffer. The bound solutes can be released by eluting the column with a buffer of increased ionic strength or pH. An increase in buffer ionic strength released bound solutes by displacement. Increasing the buffer pH decreases the strength of the interaction by reducing the charge on the solute or on the resin.

ION EXCHANGER

Ion exchanger are made up of two parts - an insoluble matrix and chemically bounded charged groups within and on the surface of the matrix. An ion exchanger is classified as cationic or anionic depending on whether it exchanges cations or anions.

Cation exchanger (also called acidic ion exchanger): it is used for cation separation.

Anion exchanger (also called basic ion exchanger) it is used for anion separation.

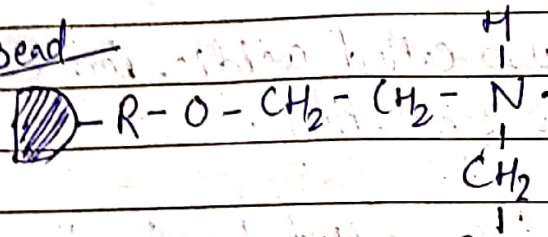
Each type of exchanger is also classified as strong or weak according to the ionizing strength of the functional group. An exchanger with a quaternary amino group is therefore, a ~~basic~~ strongly basic anion exchanger whereas primary or secondary aromatic or aliphatic amino groups would lead to a weakly basic anion exchanger. A strongly acidic cation exchanger contains the sulfonic acid group.

Anion exchanger:-

Name (Resin)	Functional group	Type
DEAE-Cellulose	Diethylaminoethyl	Weak basic
QAE-Sephadex	Quaternary aminoethyl	Strong basic
Q-Sepharose	Quaternary ammonium	Strong basic

Cation exchanger Name - Resin	Type Functional grp.	Type
CM - Cellulose	Carboxymethyl (CM)	Weakly acidic
SP - Sepharose	Sulfopropyl (SP)	Strongly acidic
Source S	Methyl sulphate (S)	Strongly acidic

Bead



DEAE : Pka = 9.5

Anion exchanger - binds to -vely charged molecules (anions)

CM : Pka = 4.0

Cation exchanger - binds to +vely charged molecules (cations)

A surface in a given sample, which has the opposite charge to that of the charged group of the ion exchanger binds to the column. Separation of charged solutes occurs because different solutes have different degree of interaction with the ion exchanger due to the difference in their charges, charge density and distribution of charge on their surfaces. If a solute has a net +ve charge at pH 7, it will

usually bind to ion exchanger (cation exchanger) containing carboxylate groups whereas a -vely charged protein will not.

(+) will attract -vely charged anions. When a mixture of solute is loaded into anion exchanger, -vely charged

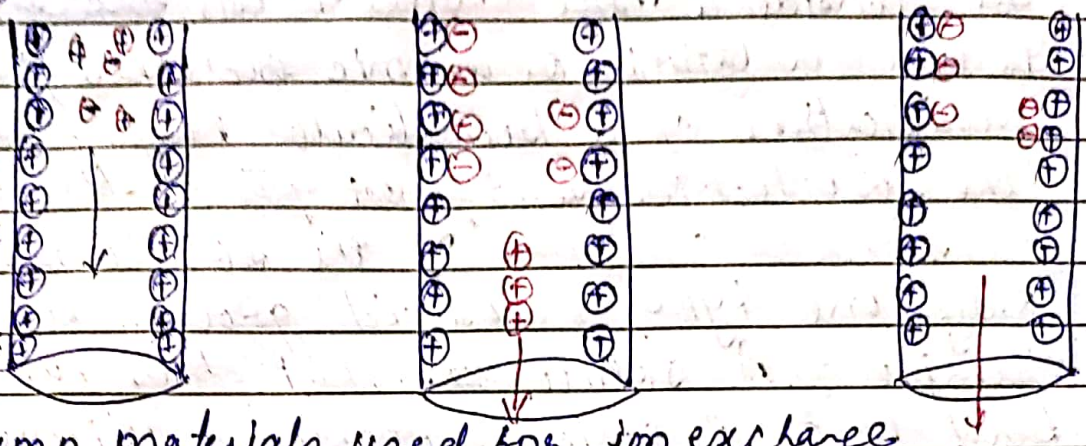


Fig:- Column materials used for ion exchange chromatography contain charged groups covalently linked to the surface of an insoluble matrix. Anion exchangers have +vely charged groups that ⊛.

Solutes bind to exchanger

The bound molecule ~~not~~ can be eluted by altering the pH of the eluting buffer or by increasing the salt concentration of the eluting buffer. A +vely charged protein ~~buffer~~ bound to cation exchanger can be eluted by increasing the salt concentration in the eluting buffer because cation present in the buffer compete with +vely charged groups on the protein for binding to the ion exchanger. Proteins that have low density to net +ve charge will ~~end~~ tend to emerge first, followed by those having a higher charge density.

Selection of Ion exchanger

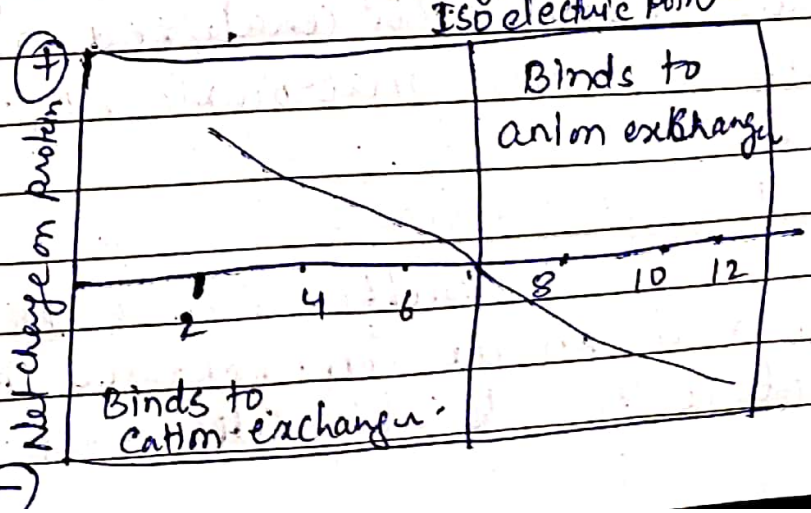
Before a proper choice of ion exchanger can be made, the nature of solutes to be separated must be considered. The choice of ion exchanger (whether to use a cationic or anionic exchanger) for the purification of a biomolecule largely depends on the isoelectric point, or pI of the biomolecule.

If the solute molecule has only one type of charged group, the choice is simple. A solute that has +ve charge will bind to a cationic exchanger & vice versa. However, many solutes have more than one type of ionizing groups and many have both -ve and +ve charged groups. The net charge on such molecules depends on pH. At the isoelectric point, the solute has no net charge and would not bind to any type of ion exchanger. At a pH value above the pI of a solute, it will have a net -ve charge and absorb to an anion exchanger. Below the pI, the solute has a net +ve charge and will absorb to a cation exchanger.

- pH > PI, net -ve charge, binds to anion exchanger
- pH = PI no net charge, no binding to ion exchanger
- pH < PI, net +ve charge, binds to cation exchanger

In principle, solutes with both -Vely and +vely charged groups should bind to both anionic & cationic exchangers. However, when one is dealing with large biomolecules, the pH range of stability must also be evaluated. The range of stability refers to the pH range in which the biomolecule is not denatured. For ex- if the pI of a protein is 4, then in most cases, it is advisable to choose an ion exchanger which binds to the protein at a pH > 4. Since, at pH > 4 this protein is -Vely charged, the ion exchanger has to be an anion exchanger eg - DEAE.

One could also use pH < 4 and a cation exchanger, but many proteins are not stable or aggregate under these conditions. If, in contrast, the protein we want to purify has a pI = 10, it is +vely charged at a pH around 7. Thus in general for this protein type we have to choose a cation exchanger, which is -Vely charged at neutral pH.



In most cases, the isoelectric point of protein is not known. The type of ion exchanger must be chosen by trial & error as follows.

→ Small samples of the solutes mixture in buffer are equilibrated for 10-15 minutes in separate test tubes, one with each type of ion-exchanger.

→ The tubes are then centrifuged to let stand to sediment the ion exchanger.

→ Check each supernatant for the presence of solute. if a supernatant has relatively low level of added solute that ion exchanger would be suitable for use. This simple test can also be extended to find conditions for elution of the desired macromolecules from the ion exchanger. The ion exchanger charged with the macromolecule is treated with buffers of increasing ionic strength or changing pH. The supernatant after each treatment is analyzed as before for release of the macromolecules.

Choice of Buffer:

Buffer ions with a charge opposite to that on the ion exchanger compete with solute for binding sites and greatly reduce

the capacity of column. Cationic buffers should be used with anionic exchangers, anionic buffers should be used with cationic exchanger.

The pH chosen for the buffer depends first of all on the range of stability of the macromolecule to be separated. Second, the buffer pH should be chosen so that the desired macromolecule will bind to the ion exchanger. In addition, the ionic strength should be relatively low to avoid damping of the interaction between solute and ion exchanger. Buffer concentrations in the range 0.05 to 0.1 M are recommended.

—:+:—