Lecture 30: Ion-Exchange Chromatography

Ion exchange chromatography: Ion-exchange chromatography is a versatile, high resolution chromatography techniques to purify the protein from a complex mixture. In addition, this chromatography has a high loading capacity to handle large sample volume and the chromatography operation is very simple.

Principle: This chromatography distributes the analyte molecule as per charge and their affinity towards the oppositively charged matrix. The analytes bound to the matrix are exchanged with a competitive counter ion to elute. The interaction between matrix and analyte is determined by net charge, ionic strength and pH of the buffer. For example, when a mixture of positively charged analyte (M, M^{+}, M^{-1}, M^{-2}) loaded onto a positively charged matrix, the neutral or positively charged analyte will not bind to the matrix where as negatively charged analyte will bind as per their relative charge and needed higher concentration of counter ion to elute from matrix (Figure 30.1).

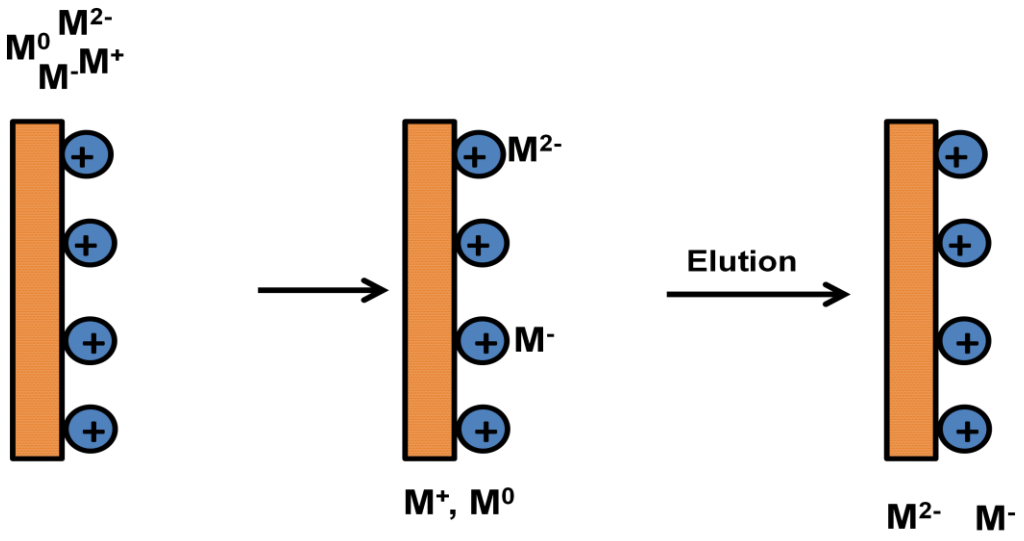

Figure 30.1: Affinity of analytes (M, M^{+}, M^{-1}, M^{-2}) towards positively charged matrix.

The matrix used in ion-exchange chromatography is present in the ionized form with reversibly bound ion to the matrix. The ion present on matrix participitate in the reversible exchange process with analyte. Hence, there are two types of ion-exchange chromatography:

1. Cation exchange chromatography- In cation exchange chromatography, matrix has a negatively charged functional group with a affinity towards positively charged molecules. The positively charged analyte replaces the reversible bound cation and binds to the matrix (Figure 30.2). In the presence of a strong cation (such as Na^+) in the mobile phase, the matrix bound positively charged analyte is replaced with the elution of analyte. The popular cation exchangers used are given in Table 30.1.

2. Anion Exchange chromatography- In anion exchange chromatography, matrix has a positively charged functional group with a affinity towards negatively charged molecules. The negatively charged analyte replaces the reversible bound anion and binds to the matrix (Figure 30.2, B). In the presence of a strong anion (such as Cl^-) in the mobile phase, the matrix bound negatively charged analyte is replaced with the elution of analyte. The popular anion exchangers used are given in Table 30.1.

Table 30.1: List of selected Ion-exchange matrix

S.No	Name	Functional Group	Type of Ion-exchanger
1	Carboxyl methyl (CM)	-OCH2COOH	Cation Exchanger
2	Sulphopropyl (SP)	-OCH2CH2CH2SO3H	Cation Exchanger
3	Sulphonate (S)	-OCH2SO3H	Cation Exchanger
4	Diethylaminoethyl (DEAE)	-OCH2CH2NH (C2H5)2	Anion Exchanger
5	Quaternary aminomethyl (Q)	-OCH2N(CH3)3	Anion Exchanger

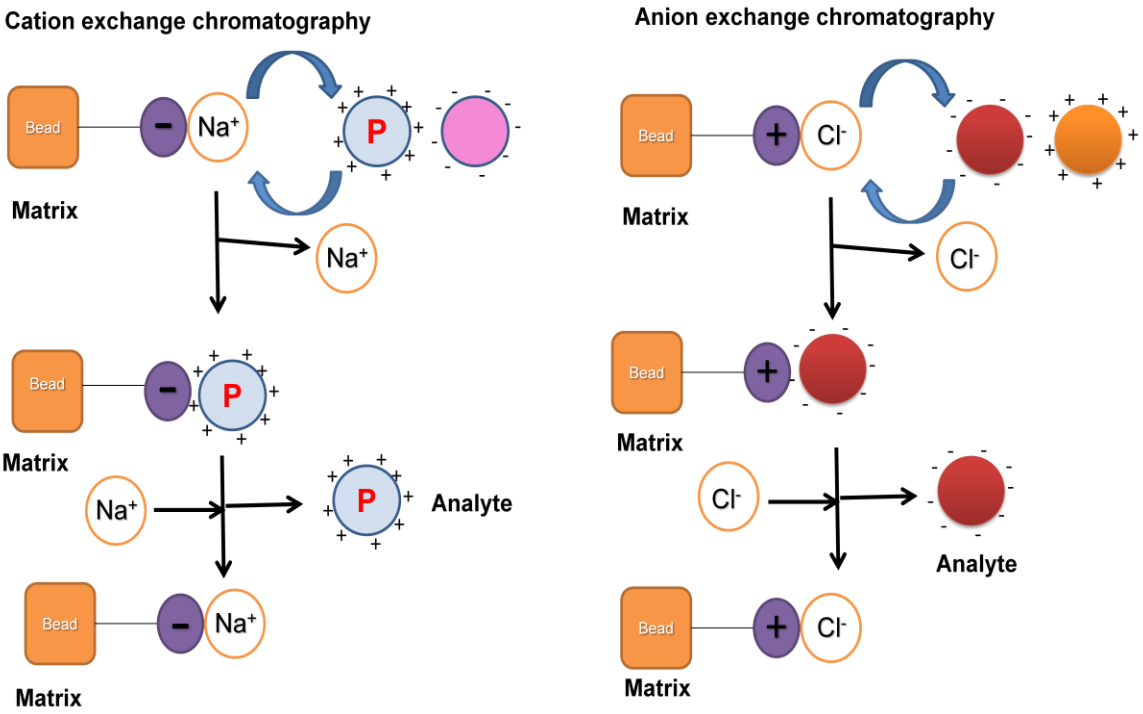

Figure 30.2: Cation and Anion exchange chromatography.

Isoelectric point and charge on a protein: Protein is a polymer made up of amino acids with ionizable side chain. At a particular pH, these amino acid side chain ionizes differentially to give a net charge (positive/negative) to the protein. The pH at which the net charge on a protein is zero is called as Isoelectric point (pI). The protein will have a net positive charge below the pI where as it has net negative charge above the pI value (Figure 30.3).

Choice of a Ion-exchange column matrix-Before starting the isolation and purification of a substance, a choice for a suitable ion-exchange chromatography is important. There are multiple parameter which can be consider for choosing the right column matrix.

1. pI value and Net charge- The information of a pI will be allow you to calculate the net charge at a particular pH on a protein. As discussed above, a cation exchange chromatography can be use below the pI where as an anion exchange chromatography can be use above the pI value.

2. Structural stability-3-D structure of a protein is maintained by electrostatic and vander waal interaction between charged amino acid, Π-Π interaction between hydrophobic side chain of amino acids. As a result, protein structure is stable in a narrow range around its pI and a large deviation from it may affect its 3-D structure.

3. Enzymatic activity-Similar to structural stability, enzymes are active in a narrow range of pH and this range should be consider for choosing an ion-exchange chromatography.

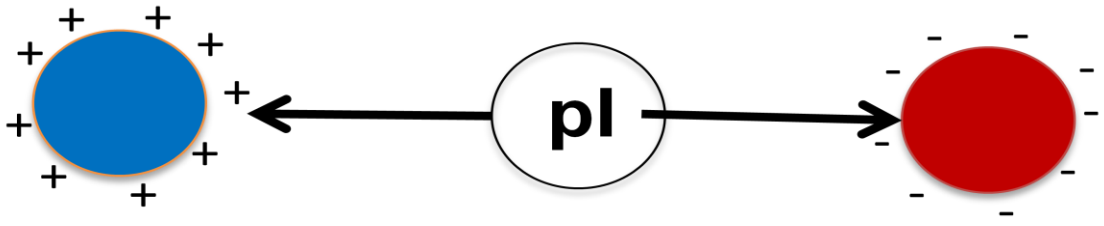

Figure 30.3: Change of charge with respect to the pI.

Operation of the technique-Several parameters needs to be consider to perform ion-exchange chromatography (Figure 30.4).

1. Column material and stationary phase-Column material should be chemically inert to avoid destruction of biological sample. It should allow free low of liquid with minimum clogging. It should be capable to withstand the back pressure and it should not compress or expand during the operation.

2. Mobile Phase-The ionic strength and pH are the crucial parameters to influence the property of the mobile phase.

3. Sample Preparation- The sample is prepared in the mobile phase and it should be free of suspended particle to avoid clogging of the column. The most recommended method to apply the sample is to inject the sample with a syringe.

4. Elution- There are many ways to elute a analyte from the ion-exchange column. (1) Isocratic elution (2) Step-wise gradient (3) Continuous gradient either by salt or pH (4) affinity elution (5) displacement chromatography

5. Column Regeneration- After the elution of analyte, ion-exchange chromatography column require a regeneration step to use next time. column is washed with a salt solution with a ionic strength of 2M to remove all non-specifically bound analytes and also to make all functional group in a iononized form to bind fresh analyte.

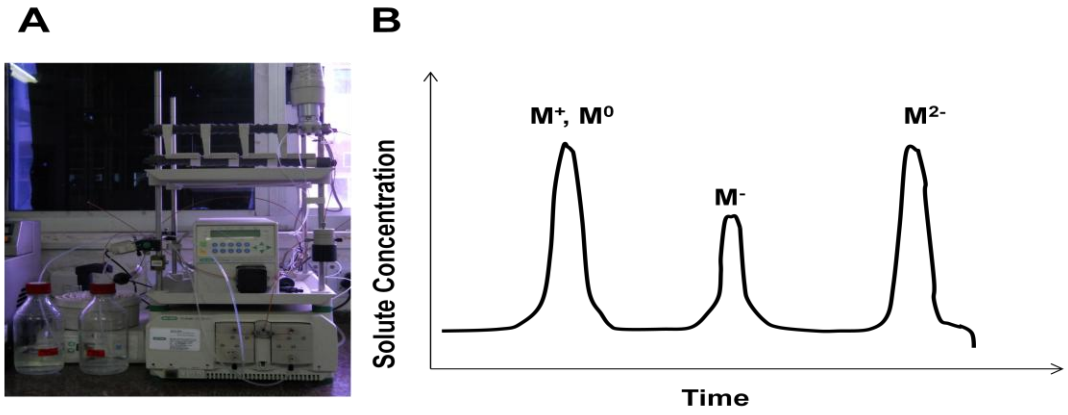

Figure 30.4 : Operation of the Ion-exchange Chromatography. (A) Chromatography system to perform gradient elution of analytes to give an (B) elution profile.