**Lec.6 *Bioseparation Technique* ion-exchange chromatography**

**Biomolecules are purified using chromatography techniques that separate them according todifferences in their specific properties, Ion exchange chromatography**

**separates biomolecules according to differences in their net surface charge.**

**Ion-exchange chromatography can be defined as the reversible exchange of ions between a liquid phase and solid phase (ion-exchange resin).Ion-exchange chromatography is commonly used to separate charged biological molecules such as protein, peptides and amino acids.**

**An Ion exchange chromatography medium comprises a matrix of spherical particles substituted with ionic groups that arenegatively or positively charged. The matrix is usually porous to give a high internal surfacearea. The medium is packed into column to form a packed bead. The bead is then equilibrated with buffer which fills the pores of the matrix and the space in between the particles.**

**Cationic ion-exchange resin normally contain a sulphonic acid, phenolic hydroxyl, carboxylic acidas active group, Carboxymethyl cellulose is a common cationic ion-exchange resin, positively charged solutes will bind to the resin, the strength of attachment depending on the net charge of the solute at the pHof the column.**

**Anionic ion-exchange resin contain amine group as active group,anion exchange resins contain bound positive functional groups,anion exchange resins are used to fractionate negatively charged proteins in a mixture, a common anionic ion-exchange resin, DEAE (Diethyl amino ethyl) - Cellulose for separation of negatively charged solutes.**

**The appropriate resin for a particular purpose will depend on various factors:**

**1- Bead size.**

**2- Pore size.**

**3- Diffusion rate.**

**4- Resin capacity.**

**5- Range of reactive groups.**

**In ion-exchange chromatography, proteins are separated based on differences in the magnitude of their charges at a given pH, So-called cation exchange resins are used to fractionate positively charged proteins in a mixture,and these resins contain bound negative functional groups.**

**With both techniques proteins having the same net charge as the resin move through the column relatively quickly. Proteins with a net charge that is opposite to that of the resin are retained, and ultimately are released by adjusting the pH or salt concentration of the elution buffer.**

**Depending on the pH, protein may carry a net positive charge, a net negative charge, or no charge, the pHof which a molecule has no net charge is called its isoelectric point (pI).**

 **In a buffer with a pH greater than the pI of the protein, the protein will carry a net negative charge; there for a positively charged anion exchange resin is chosen to capture the desired protein.In a buffer with a pH lower than the pI of the protein, the protein will carry a net positive charge, thus a negatively charged cation exchange resin is chosen.**

**If ananion exchange resin is chosen, all protein that an negatively charged at the loading buffer pH will bind to the positively charged column resin, after loading an impure protein sample onto anion-exchange chromatography column, the column is washed to removed un desired proteins and other impurities, and then protein(s) of interest is eluted using rather a salt gradient or a change in pH.**

**Protein with few charged groups will elute at low salt concentration, whereas protein with many charged groups will have greater retention time and elute at high salt concentration.**

**The protein will elute when the pH gradient reaches their pI, because will no longer carry a net charge that allows there to interact with the column resin.**

**To elute proteins from an anion exchange resin, a decreasing pH gradient is chosen, while an increasing pH gradient is chosen for elution from cation exchanger.**

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