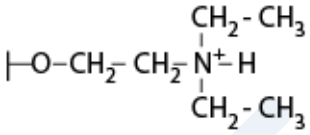
Lecture 4: Ion exchange Chromatography –II

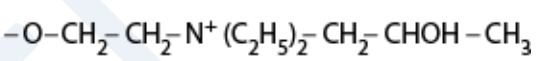
Note: This lecture is continuation of previous lecture. Before starting this one should have clear understanding of relationship of charge on protein and pH of buffer explained in last class.

Anion Exchange Chromatography: Anion exchange chromatography exploits difference in surface negative charge of protein or other molecules for separation. Anion exchange matrix has positively charged functional group. Few common examples of anion exchanger functional groups are as follows:

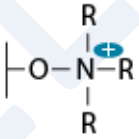
Diethylaminoethyl (DEAE)



Quaternary aminoethyl (QAE)

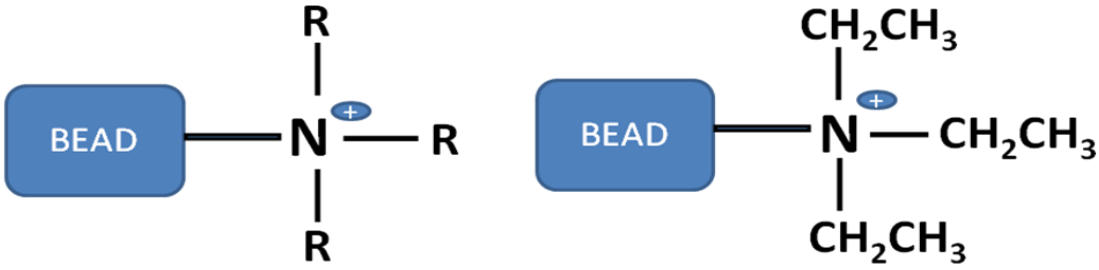


Quaternary ammonium(Q)



As explained in cation exchange chromatography, solid support with these functional groups can be prepared with various beads. They differ in few properties like flow rate etc. Anion exchangers based on dextran (Sephadex), agarose (Sepharose) or cross-linked cellulose (Sephacel) are few common options. Anion exchange Chromatography are performed using buffers at pH between 7 and 10 and running a gradient from a solution containing just this buffer to a solution containing this buffer with 1M NaCl. The surface charge of the molecule (proteins, nucleic acids etc) which bind will be net negative, thus to get binding of a specific

protein one should perform purification above the pI of that protein.



Q- Anion Exchanger DEAE Anion Exchanger

The salt in the solution competes for the binding to the column matrix and releases the protein from its bound state at a given concentration. Proteins separate because the amount of salt needed to release the protein varies with the external charge of the protein.

Method:

The amount of sample applied on the column is determined by the dimensions of column and capacity of exchanger used. After packing, column should be washed extensively with distilled water and then equilibrated with starting buffer. Once the molecules are bound, the column is washed to equilibrate it in your starting buffer, which should be of low ionic strength, then the bound molecules are eluted off using a gradient of a second buffer which steadily increases the ionic strength of the eluent solution. Alternatively, the pH of the eluent buffer can be modified as to give your protein or the matrix a charge at which they will not interact and your molecule of interest elutes from the resin. Generally gradient elution is more common than the isocratic elution. Continuous or stepwise pH and ionic strength gradient may be used but continuous gradient gives better results in comparison to stepwise. Generally for cation exchanger both pH and ionic gradient increases whereas with an anion exchanger the pH gradient decreases and the ionic strength increases.

Anion (Cl-) is attached to a positively charged matrix. Negatively charged protein competes with anion and binds to resin. A competing anion concentration (NaCl gradient) replaces the protein and elution takes place. Protein with less negative charge gets eluted early while those with more negative charge get eluted late (Fig.1 and Fig. 2)

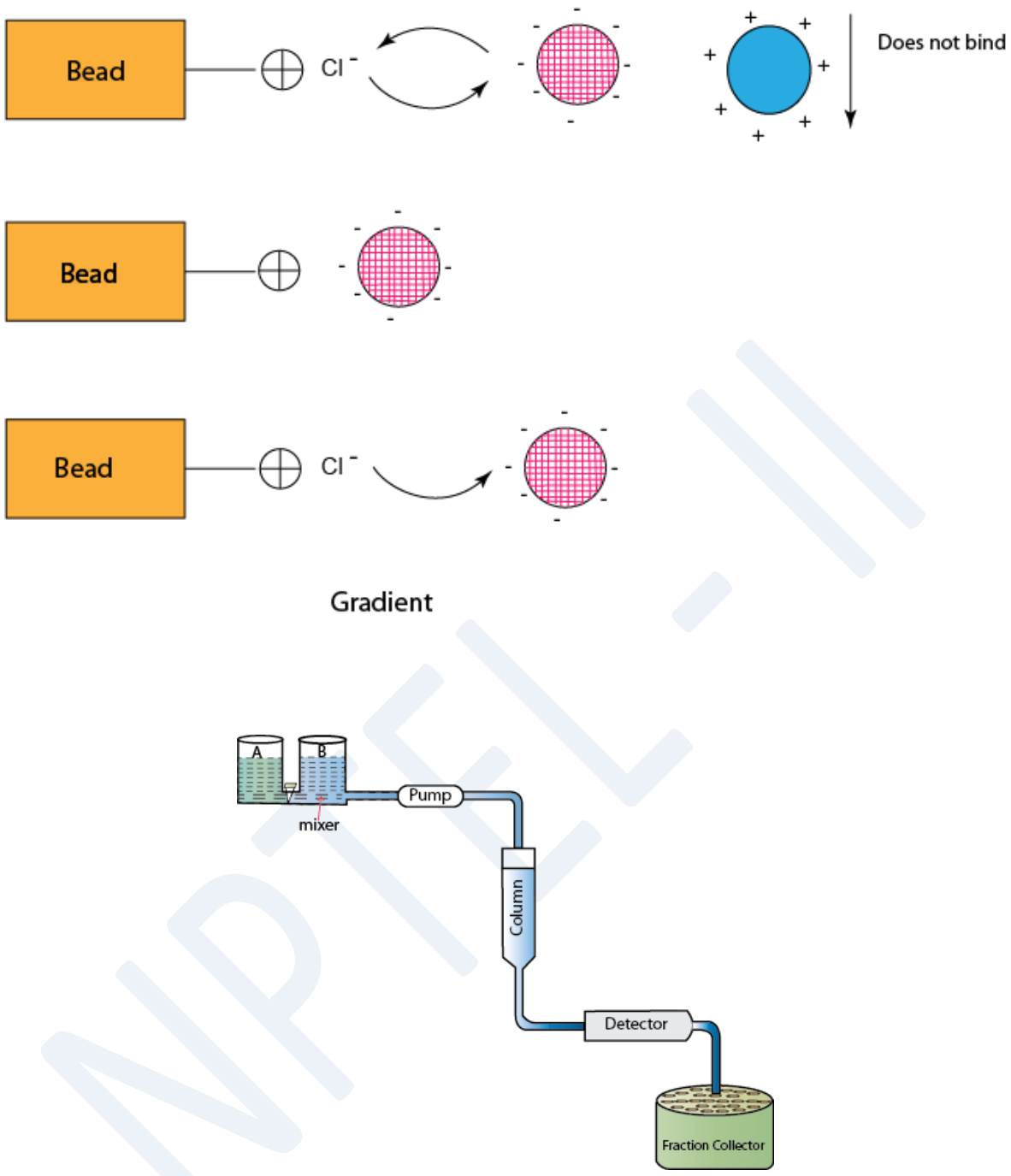


Figure 1: Expermental set-up are similar as in case of cation and anion exchange chromatography

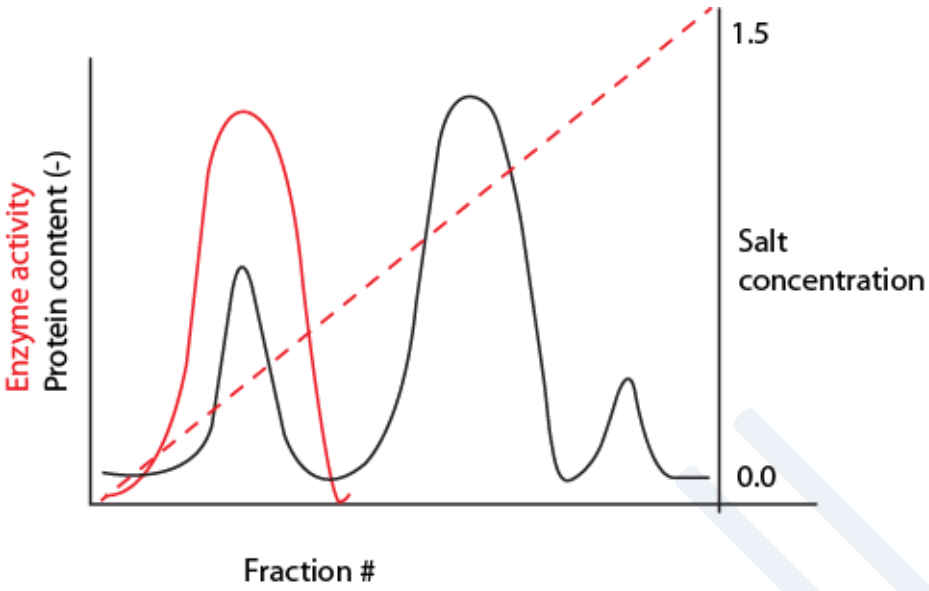


Figure 2: A typical chromatogram for an enzyme purification. First protein peak is showing enzyme activity while the second peak is not active. Thus, enzyme is purified/partially purified in the first peak. Unwanted proteins are separated in second peak. Purity of the enzyme in first peak may be checked (we shall discuss methods in coming classes). In case of partial purification, fractions covering first peak may be pooled for next step of purification.

Quiz:

- **Explain how ion exchange chromatography may be used for concentrating a protein?**