Ion exchange chromatography

Objectives:

1- The objective of this experiment is to learn the principles of ion exchange chromatography by separating the charged molecules using buffer and salt.

2- A practical experience on ion exchange chromatography in the laboratory

3- Importance of ion exchange chromatography and procedures in purification.

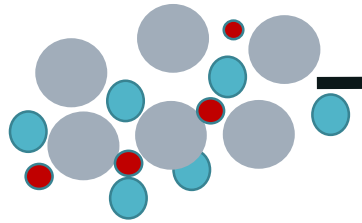
Protein Isolation and Purification

Protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms.

Whole Tissue

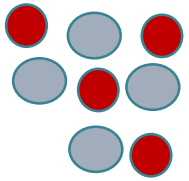
Tissue homogenization

Cell debris and other proteins are removed by centrifugation and other protein could be removed by salting out



You will have a mixture of proteins that have different charges, Sizes , and other properties

Gel filtration chromatography



You will have a mixture of proteins that have ionizable molecule similar Sizes ,

Ion exchange chromatography

- Ion exchange chromatography involves the separation of **ionizable molecules** based on their total charge.

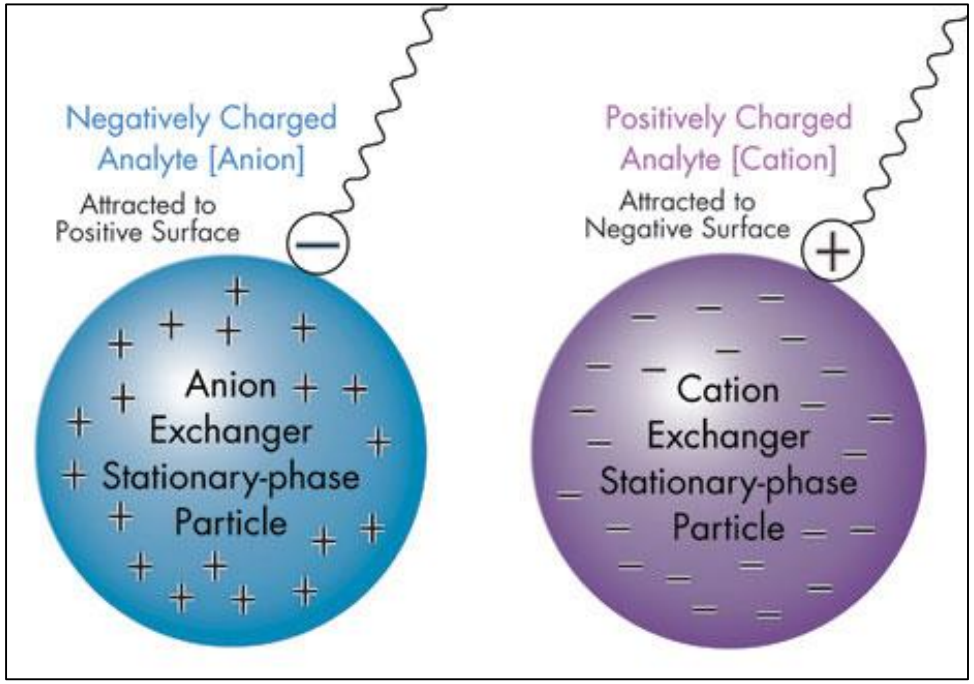
- This technique enables the separation of similar types of molecules that would be difficult to separate by other techniques because the charge carried by the molecule of interest can be readily manipulated by changing buffer pH.

Ion exchange chromatography

- Separate molecules according to charge; actually other features of the molecule are usually important so that the chromatographic behavior is sensitive to the **charge density, charge distribution**.

Types of ion exchanger chromatography

- A cation exchanger:
  - Have negative charge and **exchanges** cations
- An anion exchanger:
  - Have positive charge and **exchanges** anoins

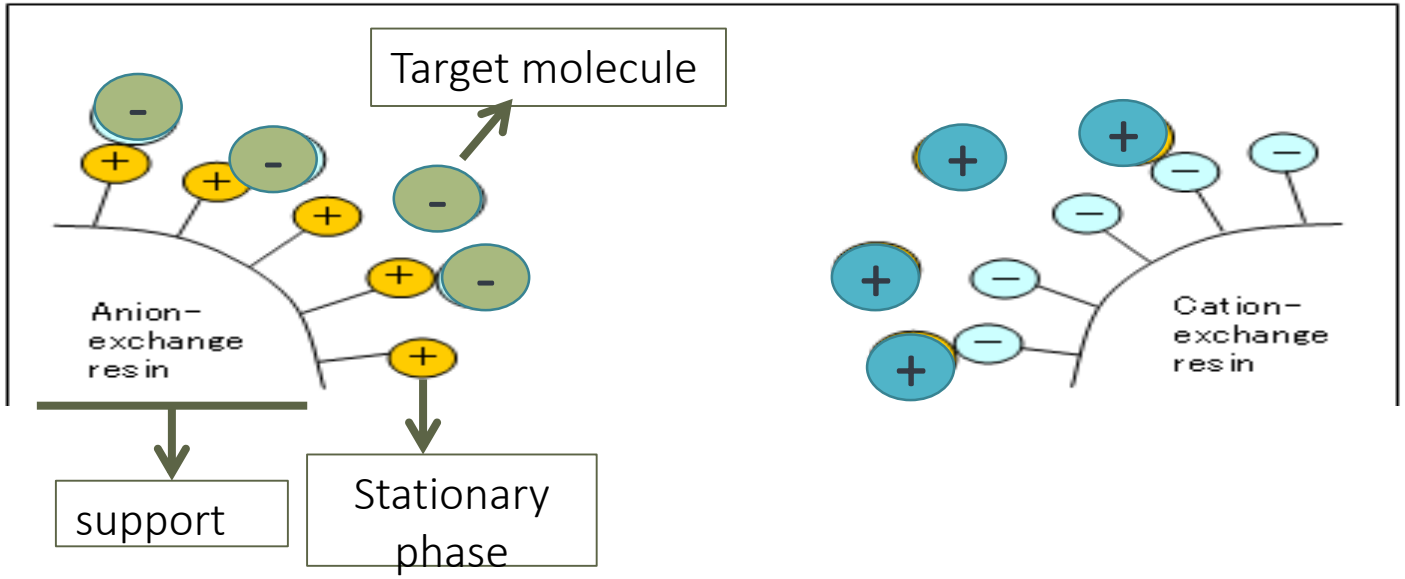


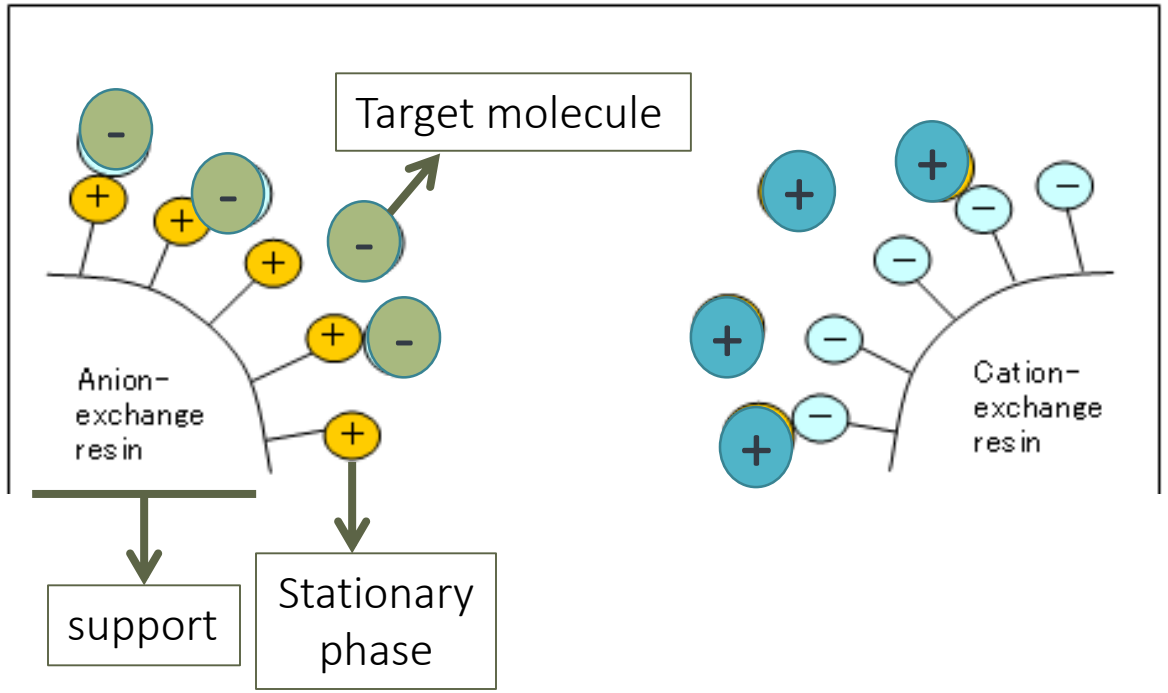
- Ion exchange chromatography is a type of adsorption chromatography
- Charged molecules adsorb to ion exchangers **reversibly** so, the molecules can be bounded or eluted by changing the ionic environment.

Stationary Phase

- Stationary phase material is a **resin or gel matrix beads** with **covalently bonded** charged functional groups. [differ from gel filtration chromatography].

- harged molecules (including proteins) bind electrostatically (non covalently) to oppositely charged groups of (stationary phase) that have been bound **covalently** on the matrix (support).





1-Support(inert): materials that can be used are dextran, cellulose, and agarose.

2-Stationary phase: is the charged groups which bound covalently to support,

- Anion exchangers have positive charge group: such as **diethylaminoethyl (DEAE),**

DEAE: matrix-CH2-CH2-N+H(CH2CH3)

Cation exchanger have negative charge group: such as **carboxymethyl group(CM)**

CM: matrix- CH_2 – COO^-

3-mobile phase: buffer or salt

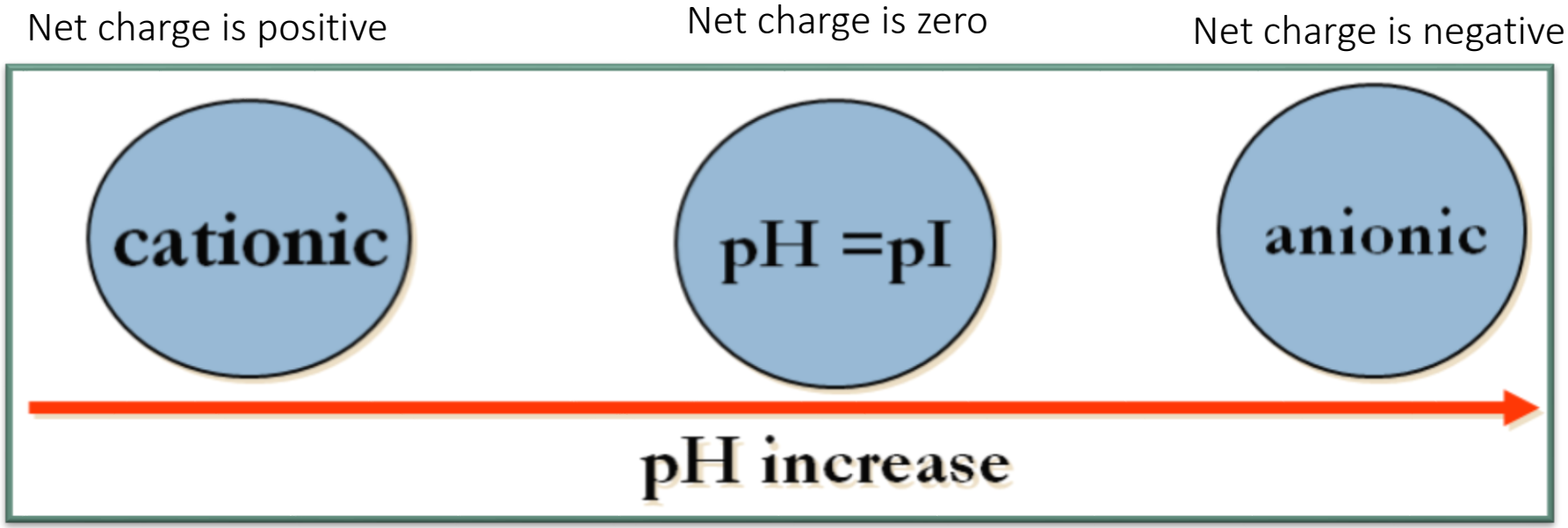
How to choose the suitable ion exchanger

- To choose whether the ion exchanger is to be anionic or cationic depend on the material to be separated.

- If the material to be bound to the column have a single charge (i.e., either plus or minus), the choice is clear.

How to choose the suitable ion exchanger

- However, many substances (e.g., proteins), carry both negative and positive charges and the net charge **depends on the pH**.



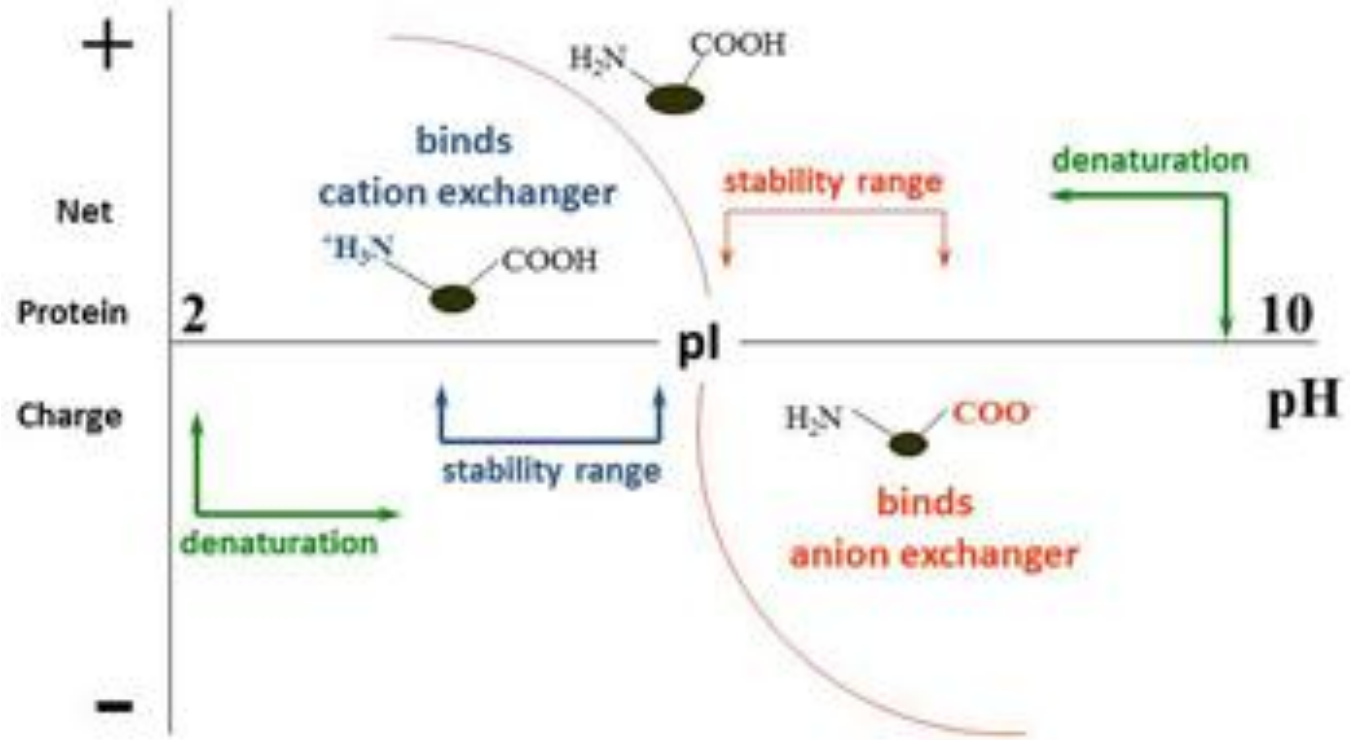
How to choose the suitable ion exchanger

- In such cases, the primary factor is the **stability** of the substance at various pH values.
- Most proteins have a pH range of stability (i.e., in which they don't denature) in which they are either positively or negatively charged.
- So, if a protein is stable at pH value above the isoelectric point, an anoinic exchanger should be used; but if stable at values below the isoelectric point, a cation exchanger is required.
- Some proteins are stable both above and below their pI. These proteins can be purified with either an anion or cation exchanger.

How to choose the suitable ion exchanger

- Also, make sure that the targeted protein that you want to be separated have different charge from other proteins
- Thus, if a protein has a negative net charge at a given pH, an anion exchange matrix should be used for its purification.

How to choose the suitable ion exchanger



http://www.bio-rad.com/en-ca/applications-technologies/liquid-chromatography-principles/ion-exchange-chromatography

Molecules Separation using ion exchanger chromatography

- **Separation on ion exchangers is usually accomplished in two stages:**

- First, the substances to be separated are bound to the exchanger by using conditions that give stable and tight binding also unwanted materials should have different charge if possible.

- Second, the target molecule then must be eluted (dissociated) either by:

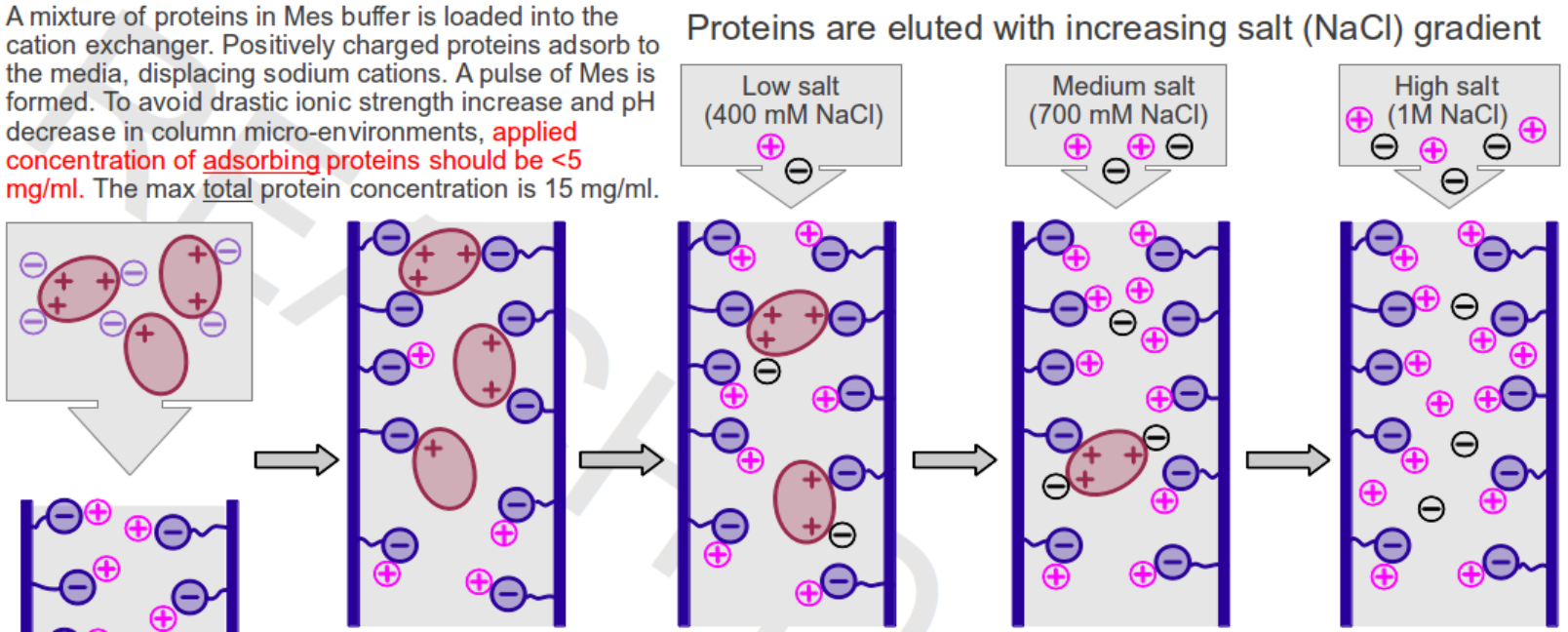
1. By applying ionic strength(eg,NaCl) so that there will be competition between the salt and target protein to bind with stationary phase
2. Or by changing pH so the charge of the protein will change

1-Example for elution of the analyte by using salts (increase ionic strength):

-In cation exchange chromatography, to elute the positively charged analyte we could elute the positively charged analyte by the addition of NaCl.

-The sodium ions[+] of NaCl, will compete with the analyte[+] to bind to the charged stationary phase[-].

-This will cause the elution of the analyte from the column.



2-Elution of the analyte by using buffers:

at a given pH a protein will possess an overall net charge.

-At pH of buffer lower than pI of the analyte , the net charge will be more positive.

-At pH of buffer higher than pI of the analyte , the net charge will be more negative.

So, we can change the net charge of the analyte in a way that it will elute from the column if it has the same charge of the stationary phase, by using the suitable pH of the buffer and knowing the pI of the analyte.

Or by using buffer has pH that will cause the net charge of the analyte equal to zero[no charge], leading to the analyte to be eluted.

Practical

Practical:

-You are provided with Cation exchange chromatography column, separate the sample given to you.

-The sample is a mixture of two proteins, myoglobin and cytochrom C,[pI=7.2 and 10.2 respectively] dissolved in phosphate buffer[pH=8]. Separate the mixture by using the buffer and 1M NaCl.

1-carefully remove the layer of the phosphate buffer from above the resin using a pasture pipette, and let only a very thin layer of buffer.

2- Again using the pasture pipette, very slowly layer the sample mixture solution on the top of the resin, by adding the tip of pipette on the wall of column. Care should be taken not to disturb the gel beads.

3- Open the screw clip, and start to collect the fractions of about 3 ml each.

4- After the sample mixture penetrates the gel so that you can see the gel beads, carefully fill up the column with the phosphate buffer [pH=8] , and complete the collection of fractions.

5-Collect the fraction of the first sample, and make sure that it is completely eluted.

6-start adding the 1M NaCl to elute the second sample, and collect the fraction, then make sure that it is eluted completely.

7- Read the absorbance of each fraction at 410 nm by using spectrophotometer using phosphate buffer as blank for the fractions was collected by using the buffer to elute them, and use 1 M NaCl as a blank for the fractions was collected by using the salt.

8-plot the curve[absorbance at 410nm against fraction number].

Resin Type	Cation Exchanger	Anion Exchanger
Net charge of molecule of interest	+	−
Charge of resin	−	+
Running conditions	0.5–1.5 pH units below the pI of the molecule of interest	0.5–1.5 pH units above the pI of the molecule of interest

http://www.bio-rad.com/en-ca/applications-technologies/liquid-chromatography-principles/ion-exchange-chromatography