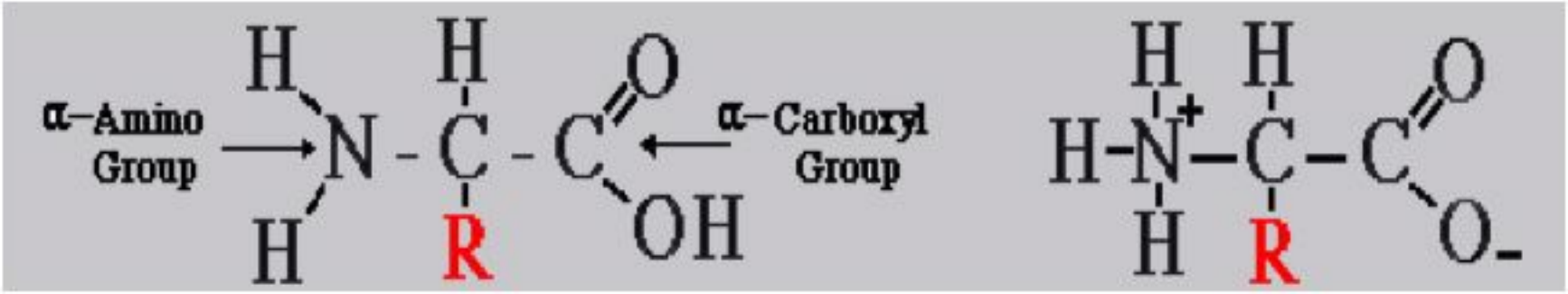
Ion Exchange Chromatography

Applications for protein purification

Ion Exchange Chromatography

- ***Widely used in industry***
- ***High capacity***
- ***Economical***
- ***Versatility in modes***
- ***Several forms available***

Amino acids on a protein can donate or accept Protons

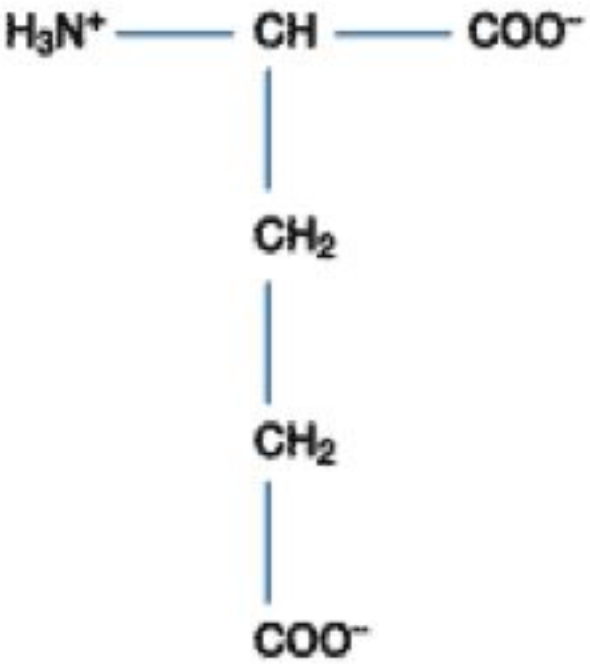


At basic (high) pH proteins give up H+ protons

At acidic (low) pH proteins accepts H+ protons

What is the net charge in this example?

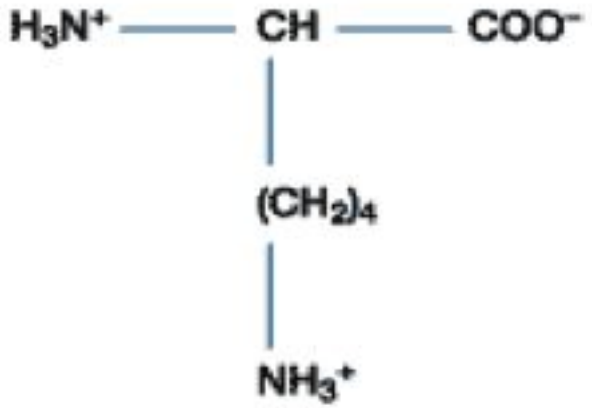
Glutamic acid : carboxyl side chain



High pH [H+] is low end group readily donates H+: lends negative charge to molecule

<u>Negative</u> charge binds to <u>Anion</u> exchanger

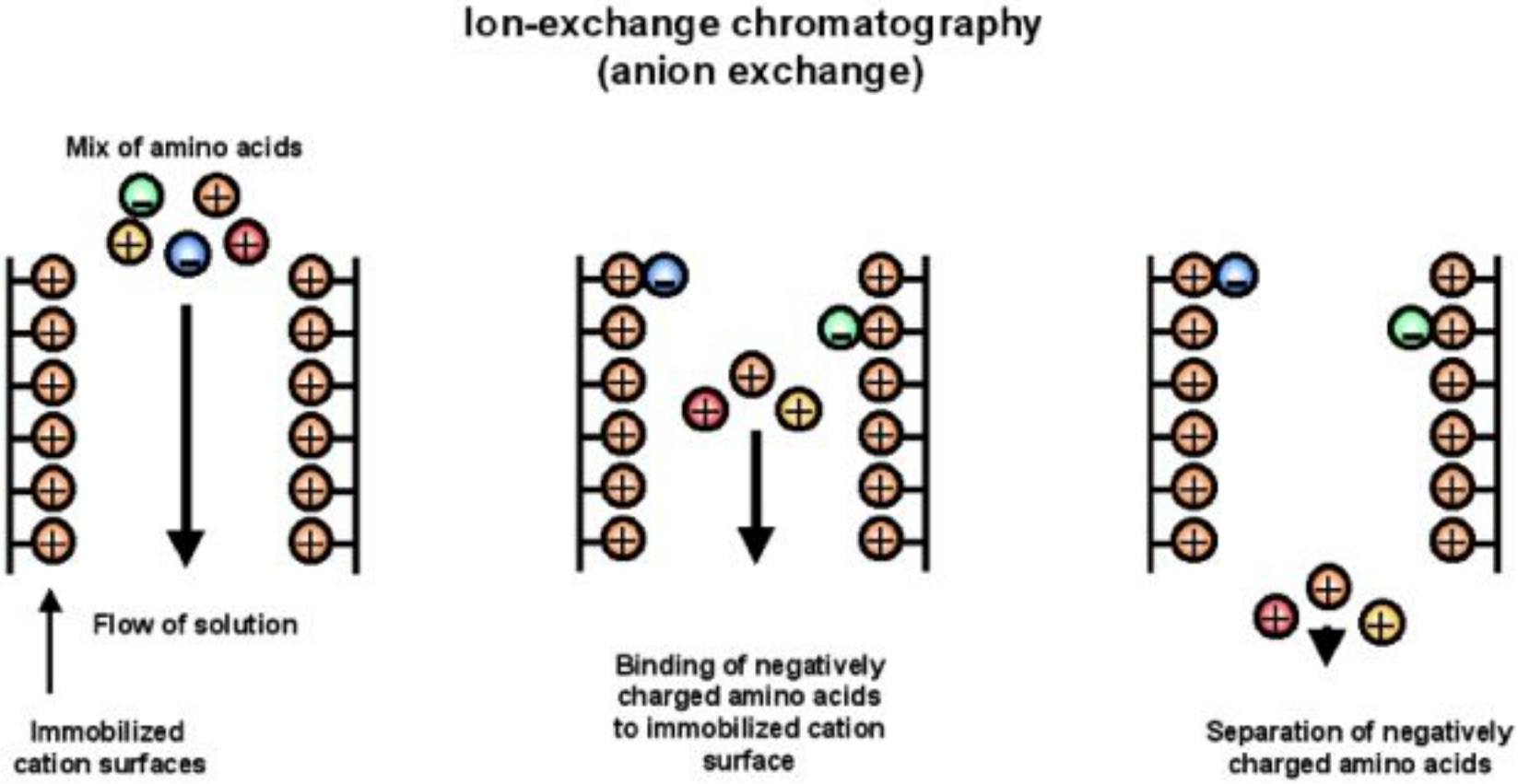
Lysine : side chain Amine group



In low pH [H+] is high
NH_2 group will accept proton to become NH_3+ lending a positive charge

Binds to a Cation exchanger

Anion exchanger - binds negatively charged molecules



Ion –exchanger Principles

Binding is dependant on the charge property of the molecule of interest

Cation exchanger

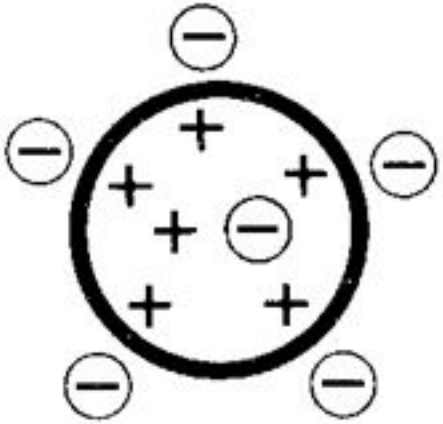
$$\underset{\text{Exchanger}}{RSO_3^-}\ \ldots\ \underset{\text{Counter ion}}{Na^+} + \underset{\text{Charged molecule to be exchanged}}{\overset{+}{N}H_3R'} \rightleftharpoons RSO_3^-\ \ldots\ \underset{\text{Bound molecular ion}}{\overset{+}{N}H_3R'} + \underset{\text{Exchanged ion}}{Na^+}$$

Anion exchanger

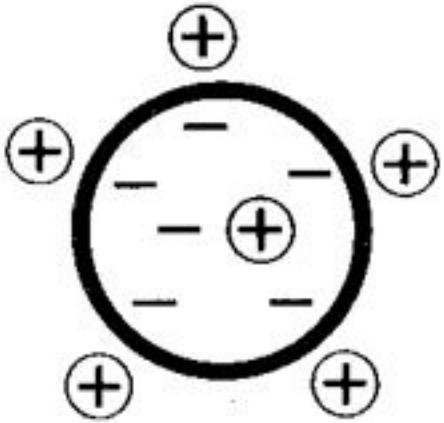
$$(R)_4\overset{+}{N}\ \ldots Cl^- + {}^-OOCR' \rightleftharpoons (\overset{+}{R})_4N\ldots{}^-OOCR' + Cl^-$$

The more highly charged the molecule to be exchanged, the tighter it binds to the exchanger and the less readily it is displaced by other ions;

Types of exchangers



ANION exchanger with exchangeable counter ions



CATION exchanger with exchangeable counter ions

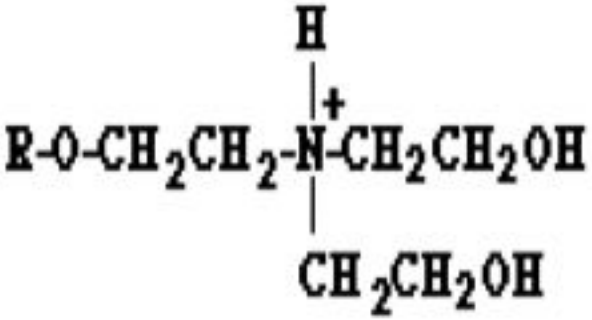
Fig. 2. Ion exchanger types.

- **Strong Ion Exchangers:** based upon strong acids or bases and are charged over a wide range of pH
- strong cation exchangers: Sulfonic Acid (or derivatives) R-SO3-
- strong anion exchangers: quaternary ammonium salts R-N (CH3)+

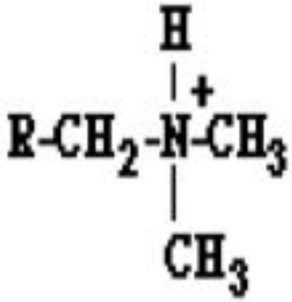
Weak Ion Exchangers: based upon weak acids or bases which are charged only over a limited pH range

- **weak anion exchangers: Diethyl-amino-ethyl (DEAE); tertiary amines**
  weak cation exchangers: carboxy methyl (CM); phosphoryl

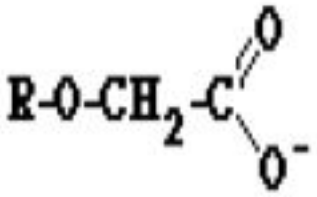
These may be bonded to a variety of supports: e.g. DEAE-cellulose; DEAE-Sephadex; DEAE-Sepharose; CM-Cellulose; CM-Sephadex; CM-Sepharose



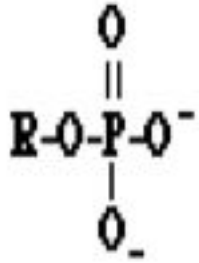
DEAE pKa = 9.5



Tertiary Amine



Carboxyl Methyl (CM) pKa = 4.0



Phosphoryl pKa's = 1.5, 6.0

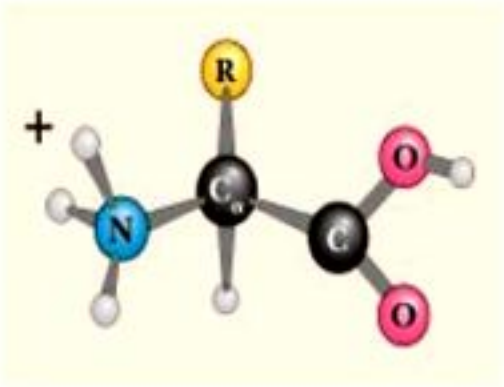
Nomenclature of some common exchangers

Cation exchanger	Type	Structure
Sulphopropyl, SP	Strong	$-(CH_2)_3SO_3^{\ominus}$
Phospho, P	Intermediate	$-H_2PO_4^{\ominus}$
Carboxymethyl, CM	Weak	$-CH_2COO^{\ominus}$
Anion exchangers	**Type**	**Structure**
Aminoethyl, AE	Weak	$-C_2H_4-N^{\oplus}H_3$
Diethylaminoethyl, DEAE	Weak	$-C_2H_4-N^{\oplus}H(C_2H_5)_2$
Quaternary aminoethyl, QAE	Strong	$-C_2H_4-N^{\oplus}(C_2H_5)_2C_3H_6OH$

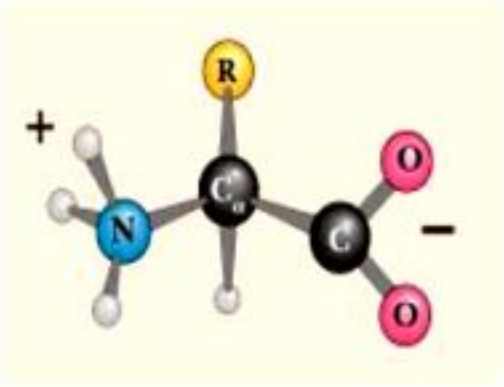
Table 2. The most common functional groups used in ion exchange chromatography

Isoelectric Point (pI)
pI = pH at which the net charge is Zero

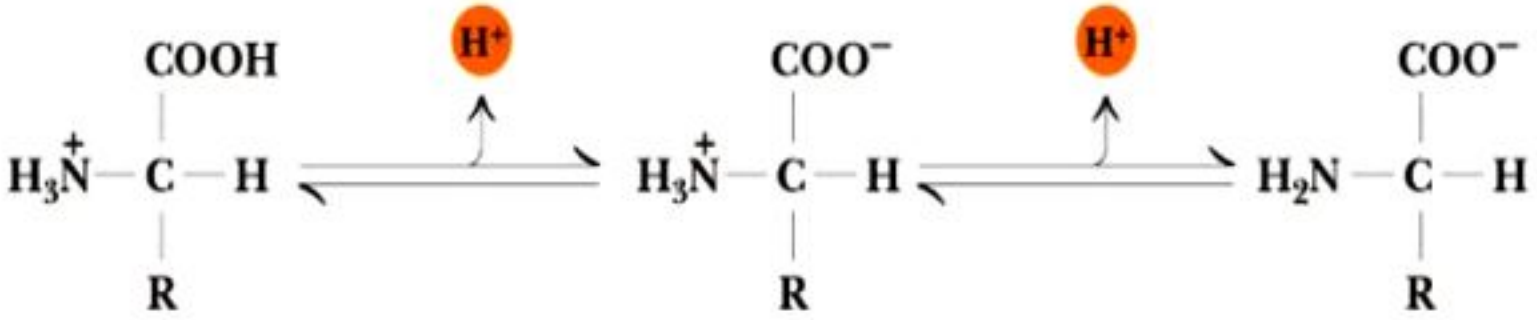
pH 1 Net charge +1



pH 7 Net charge 0



pH 13 Net charge –1



Selecting the pH range for your protein

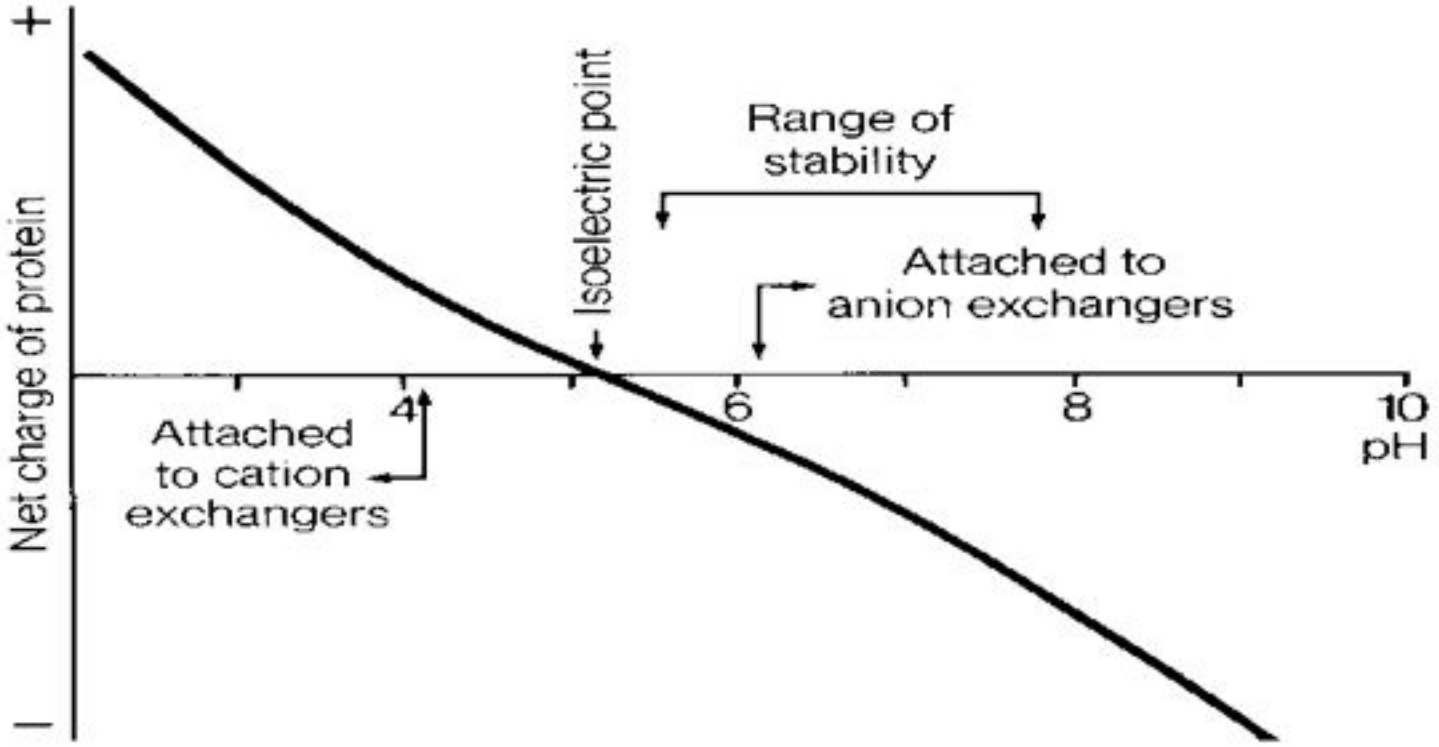


Fig. 24. The net charge of a protein as a function of pH.

Shift pH <u>2 units</u> from pH for fully functioning charge.

Example: Choosing what pH and exchanger to use for Albumin

- Given that the pI isoelectric point of Albumin is 5.1
- At pH 8.0 Albumin would be negatively charged
- An anion exchanger would bind Albumin at pH 8.0

- At pH 4.0 Albumin would be positively charged
- A cation exchanger would bind albumin at pH4.0

pH elution - mechanism

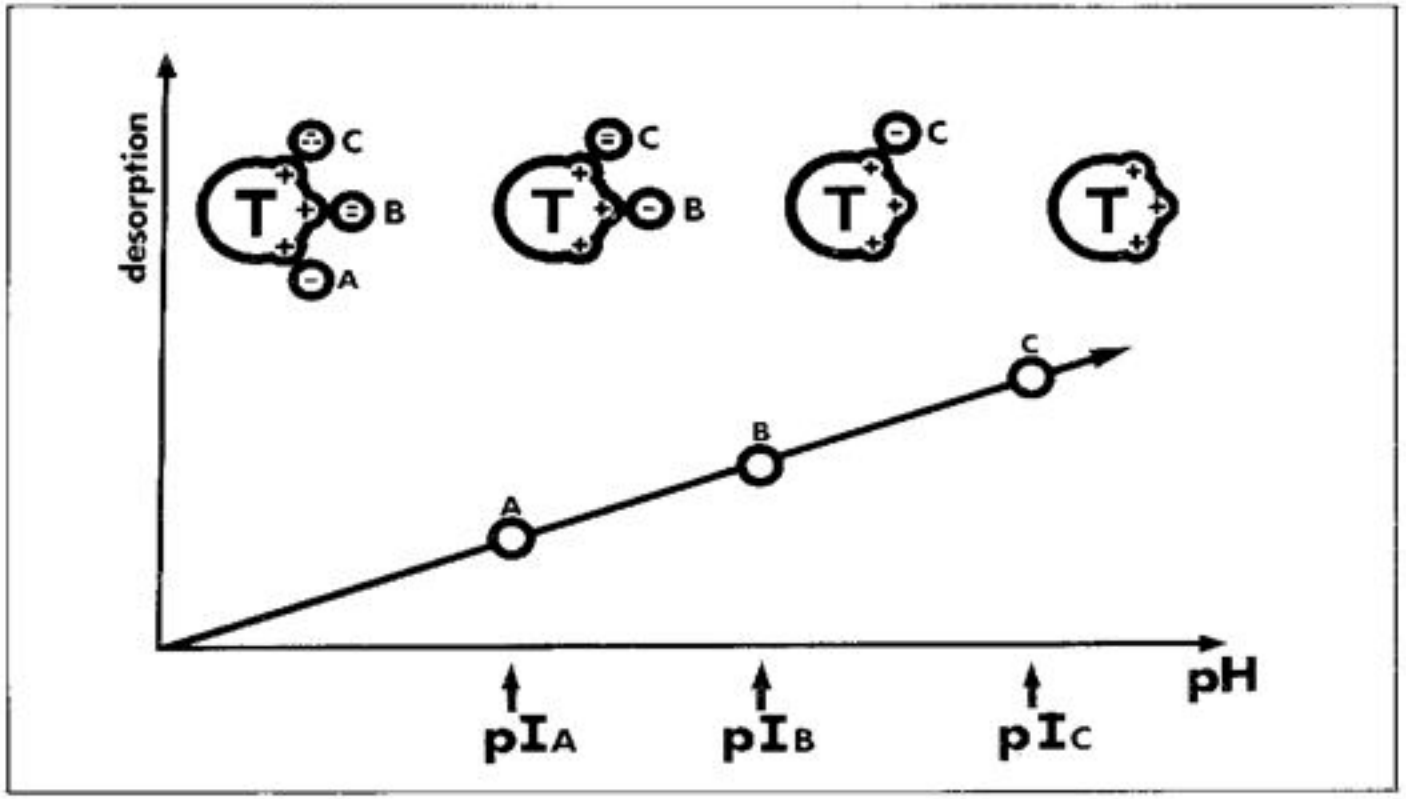


Figure 12. Elution of three different molecules (A, B and C) as a function of pII.

- ***As pH increases (or decreases) the proteins become less charged as the mobile phase matches the Isoelectric point of the proteins***

Selecting Binding conditions

- ***Determine optimum pH***
- ***Determine optimum Ionic strength***
- ***Determine capacity***

Shading denotes presence of protein!

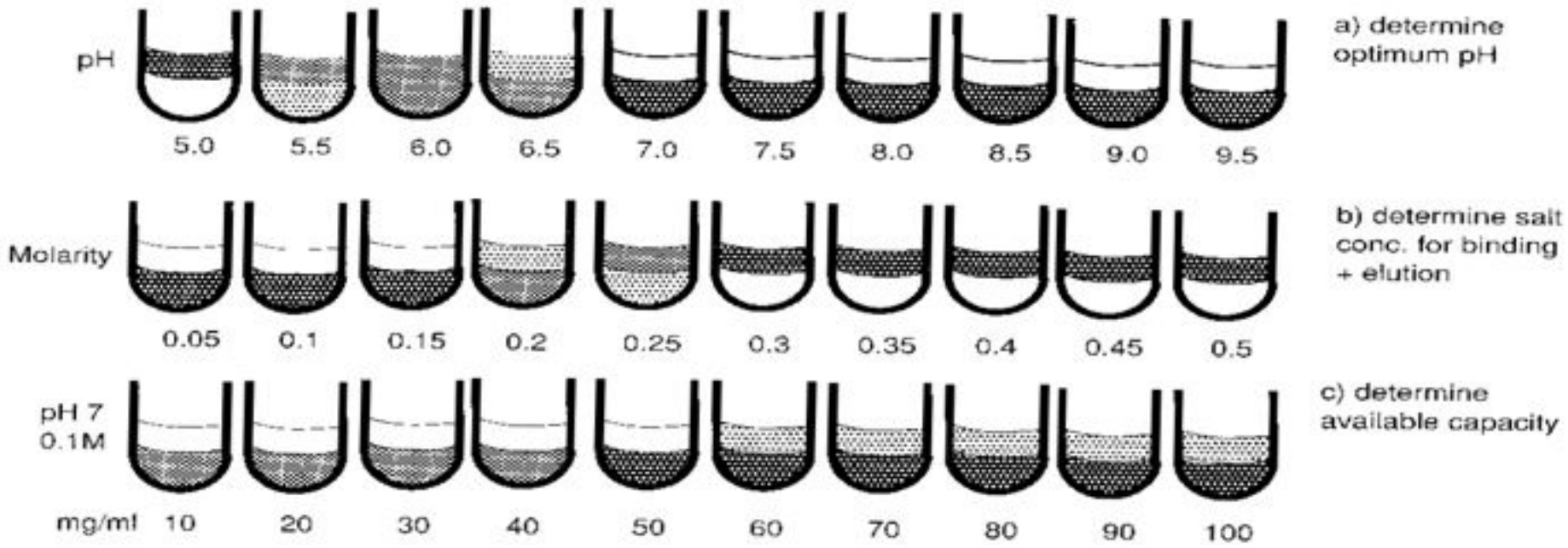


Fig. 25. Test-tube methods for selecting ion exchange conditions.

Gradient /Step elution

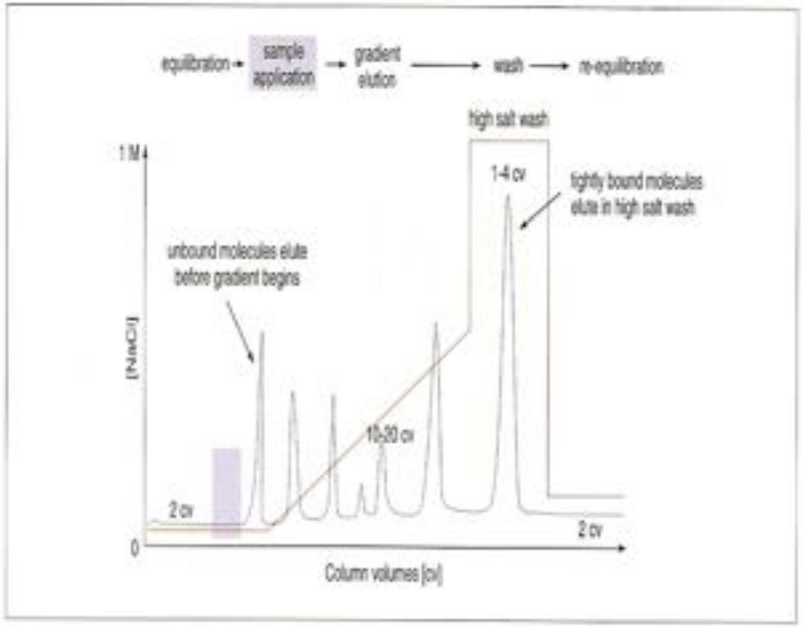


Fig. 31. Typical IEX gradient elution.

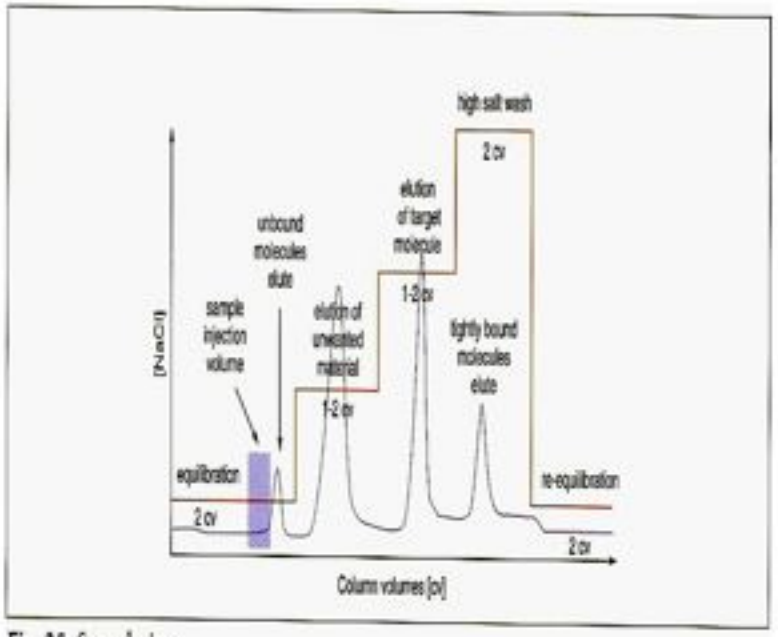
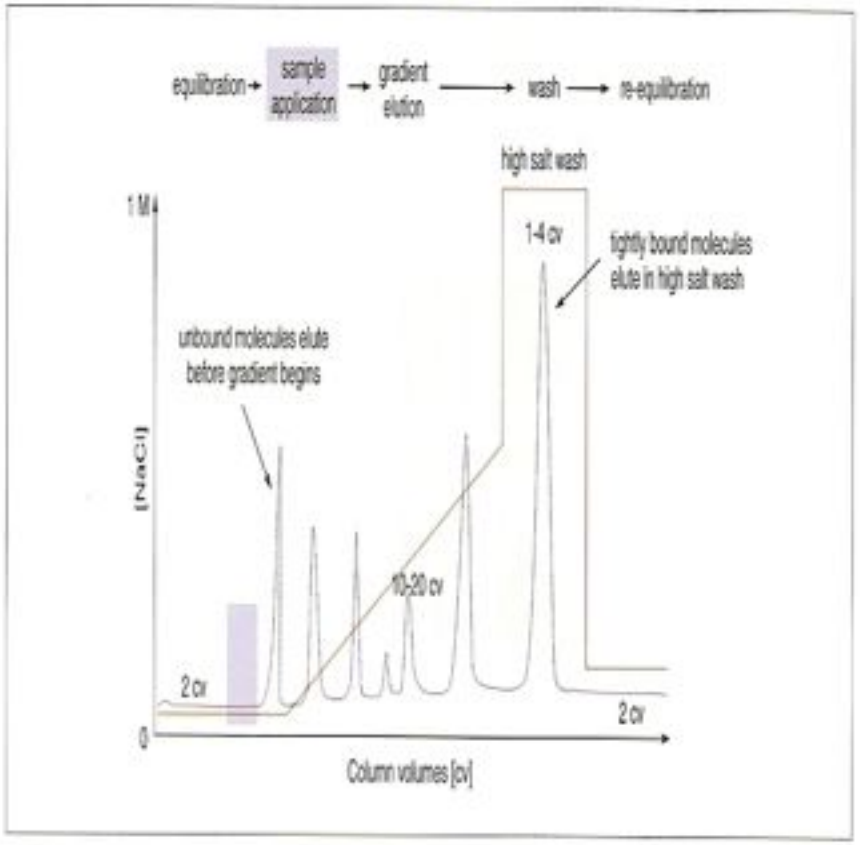


Fig. 35. Step elution.

Gradients are used for analytical ,and process development

Steps are used for larger processes: saves time and buffer volume

Typical Chromatography Profile



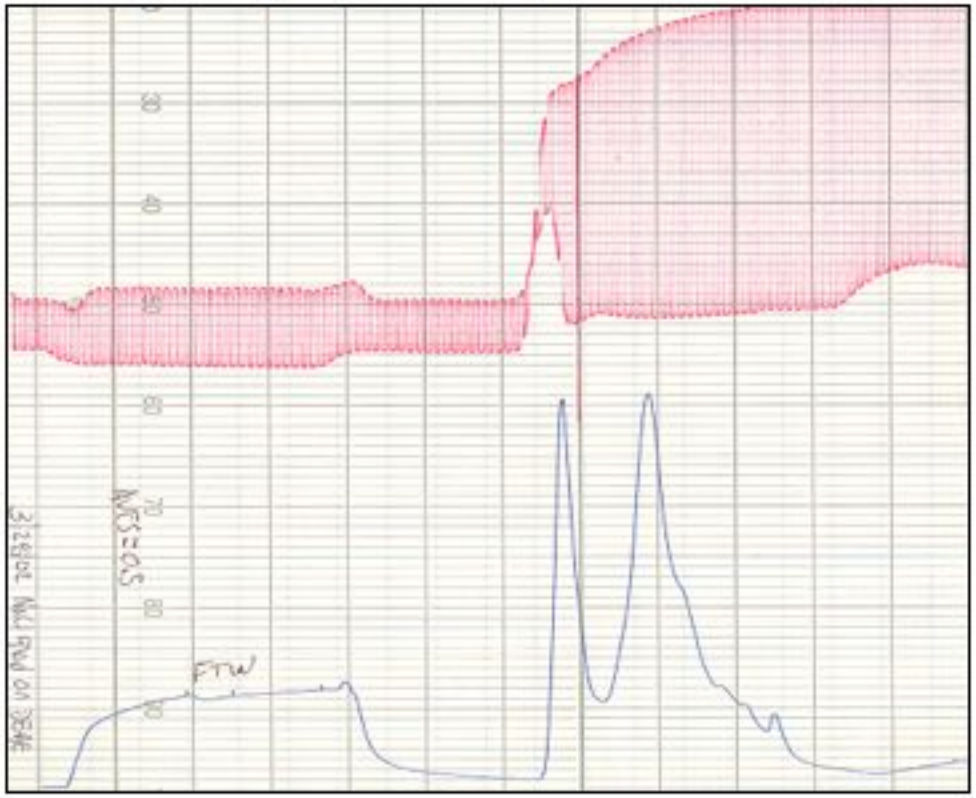


Fig. 31. Typical IEX gradient elution.

Elution by Gradient
increase resolution by making the gradient shallower

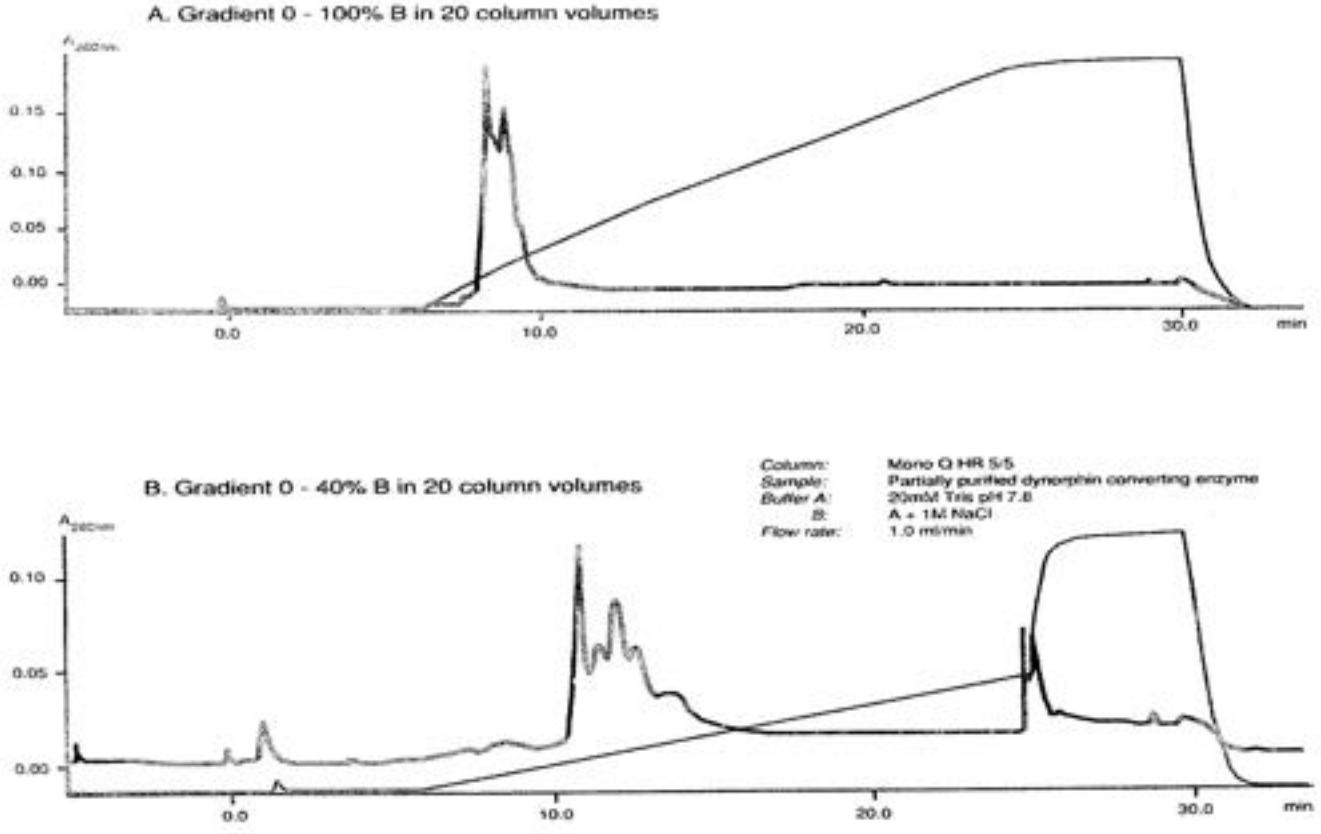


Fig. 39. Effect of gradient slope on resolution. (Work from Pharmacia, Uppsala, Sweden.).

Elution can also be effected by pH Change

Shifting the pH towards the pI of a substance causes it to lose its net charge, desorb, and elute from the ion exchanger.

Change of pH

As shown in Figure 24, the net charge on a molecule depends on pH. Thus altering the pH towards the isoelectric point of a substance causes it to lose its net charge, desorb, and elute from the ion exchanger. Figure 36 shows use of a decreasing pH gradient in separation of haemocyanin fractions (26).

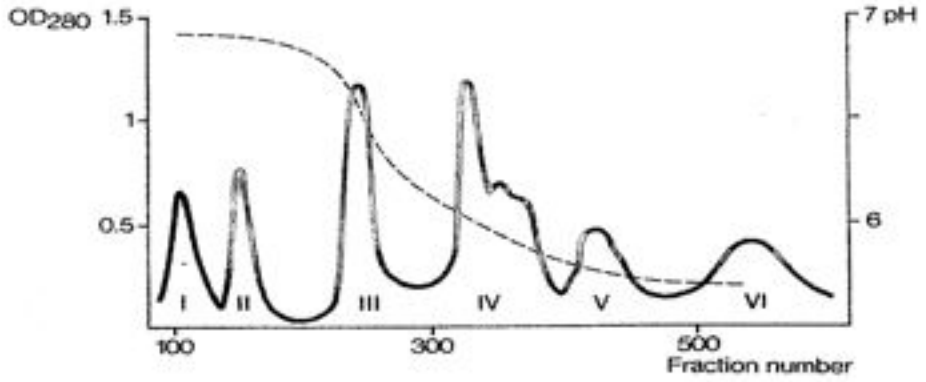
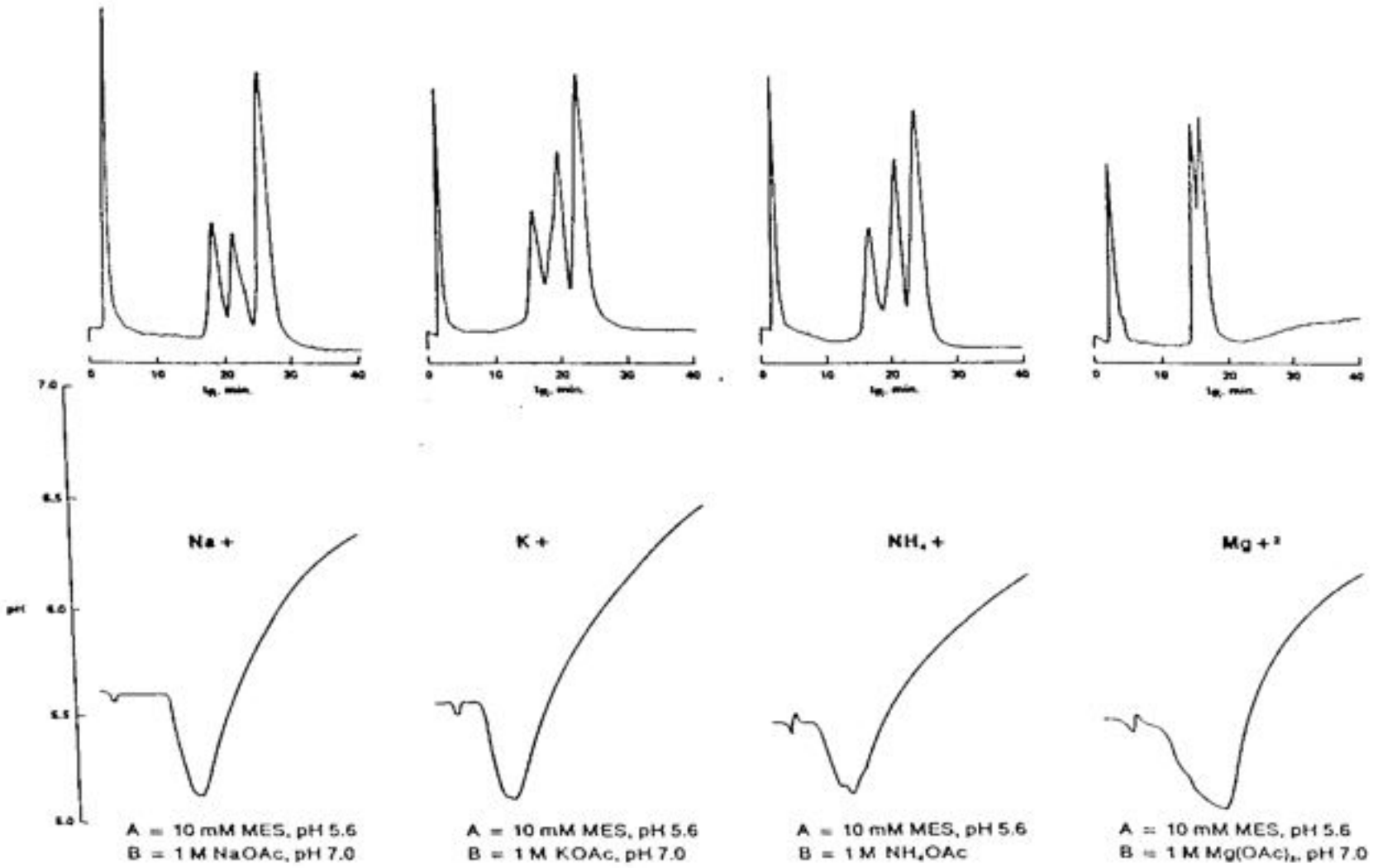
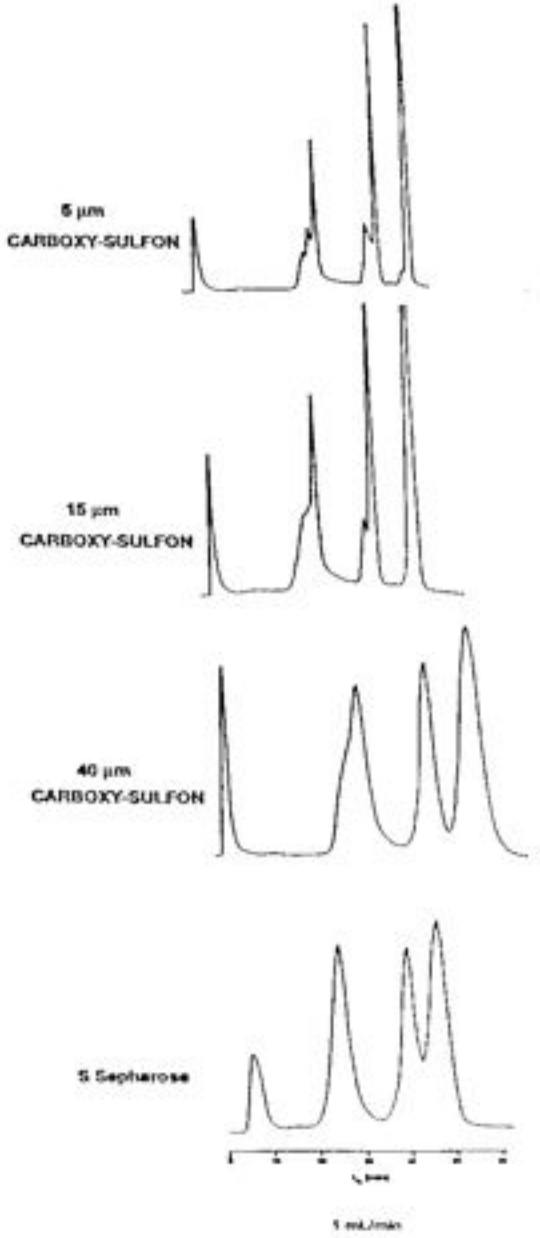


Fig. 36. Elution pattern of whole stripped haemocyanin on DEAE Sepharose CL-6B. Sample applied in 0.1 M sodium phosphate buffer pH 6.8 and eluted with decreasing pH gradient. (Lamy, J., Lamy, J., Weill, J. Arch. Biochem. Biophys. 193 (1979) 140-149. Reproduced by kind permission of the authors and publisher).

Choice of Counter-ion will affect resolution





Particle size Effect

- *Smaller columns such as HPLC can use smaller particles providing higher resolution*
- *Larger columns require larger particles for structural reasons (pressure constraints) provide less resolution*