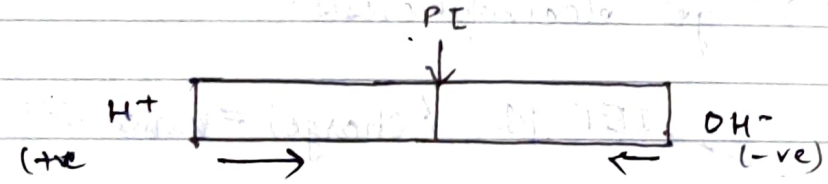
Polyacrylamide gel
↓ Two types
(i) Native gel
(ii)

* Pulse Field Electrophoresis * (2-D electrophoresis)

IEF — Iso electro focusing.

This is the name of technique. As the name indicates - the protein molecules is focussed at the pH of medium.

- Molecules have both (+ & -) charges but the net charge decides the mobility of molecule in ele field.
- When PI value of protein matches with pH of medium then only it is focussed. At this pt. we can see the molecule.



- If net charge on molecule is +ve, it will move towards -ve electrode end and vice-versa. This is the basic principle of pulse field electrophoresis.

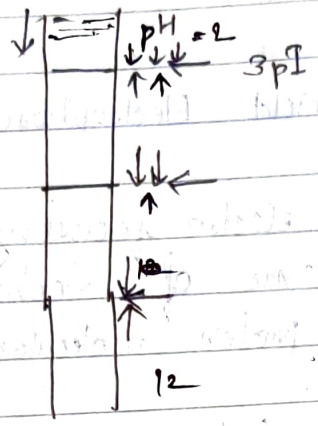
Amphdytes: It is weak electrolyte (a mixture of components acidic and basic) as the name indicates. They are very small a. acids with both charges.

- These amphdytes are mixed with medium.

pH of amphdyte.

Ampholyte — pH 2-12
pH 2-3

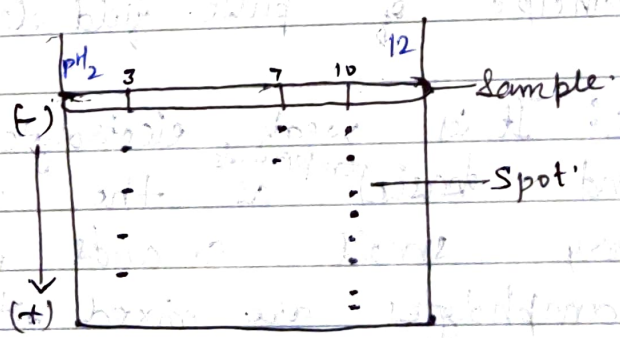
- When gel polymerisation occure and ele. field is applied then these charges of ampholyte moves and we get the gradient of pH.



Two parameters of 2-D gel electrophoresis

Using 1-D electrophoresis, proteins can't be separated on isoelectronic pH level but by 2-D i.e IE focusing. So, 2 parameters are required in 2D gel electrophoresis.

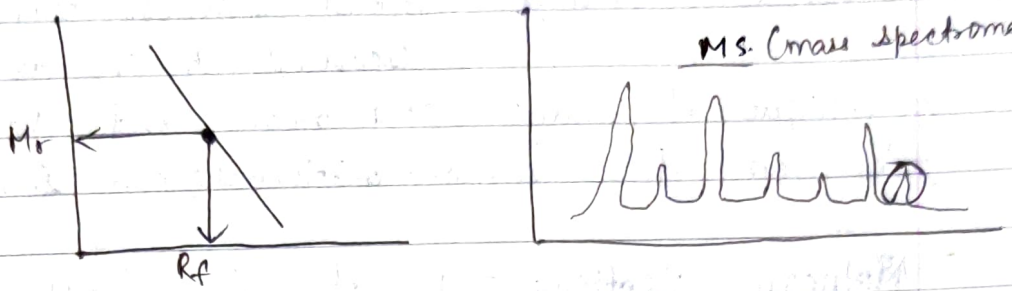
2-D
- IEF pI (charge) ≡ varies with pH.
- SDS-PAGE (mass)



When these proteins are separated they are stained by Commasie or silver stain.

IPG — Immobilised pH gradient

- Protein markers are used in side to locate the position of moving protein molecules.
- We'll get fragment k/a cryptic fragments.



Application —

1. In study of genome and chromosome.
   eg yeast cell, drosophila, mammalian cell brain cell etc.
2. In protein biochemistry — characterisation.
3. It can produce monoclonal antibodies
4. Fn. of mitochondria, chloroplast and ER could be known.
5. Protein — protein interaction.

Protein → Characterization
Antibody
Protein — protein interaction.

IP - Immunoprecipitate.

*Importance of this has been increased from — Human Genome Project (1990) implication.