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Programme: M.Sc., Biochemistry

Course Title: Analytical Biochemistry

Course Code:BC103CR

Unit IV

High voltage electrophoresis & Isotachophoresis

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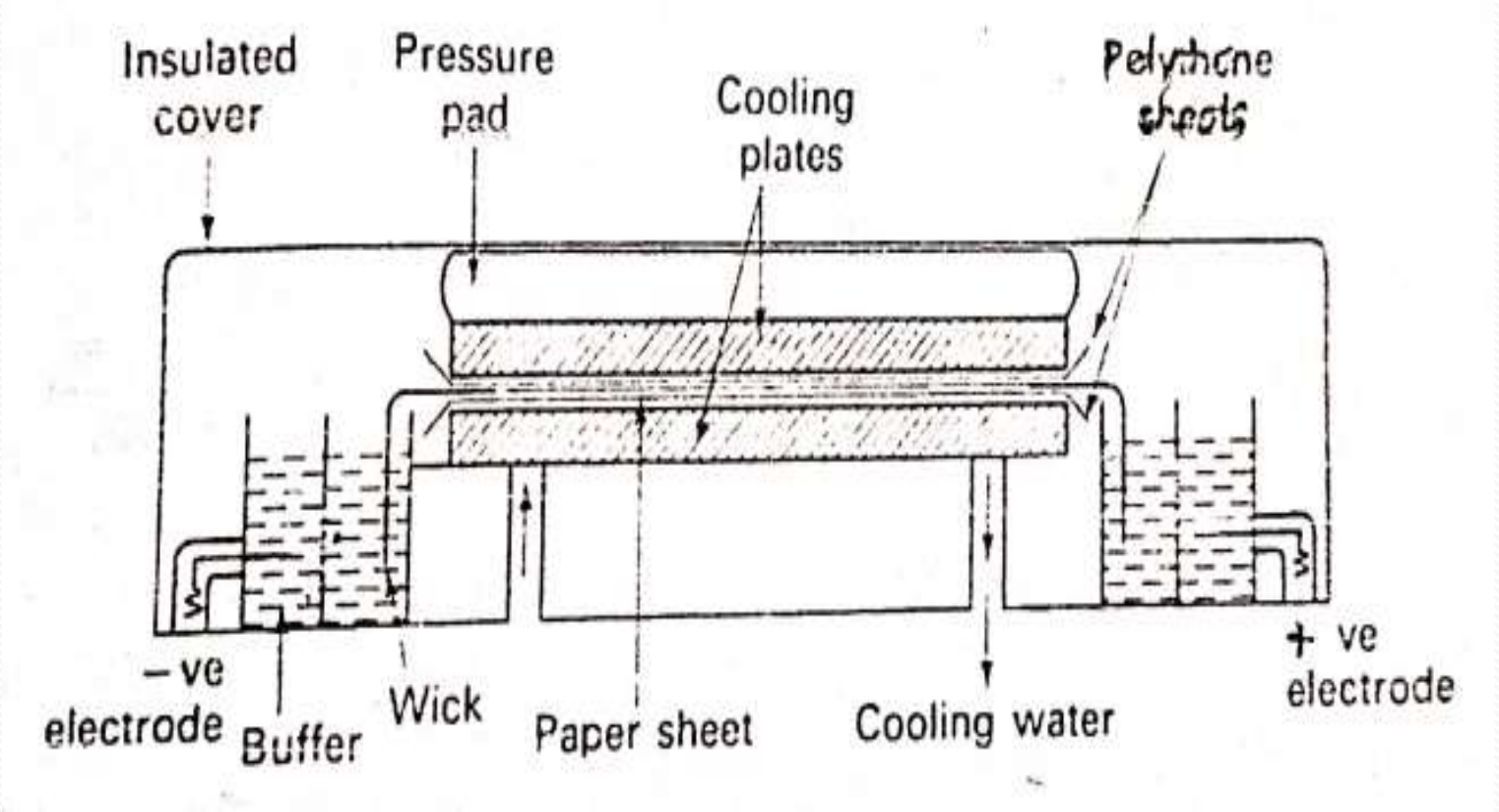
High Voltage electrophoresis (h.v.e)

- **Principle:** Charged ion or molecule migrate when placed in an electric field. The migration rate is depends on its charge, size, shape and the applied electric field. When performing electrophoresis using low voltage for the separation of low molecular weight compounds, diffusion of sample occurs. This can be overcome by applying high voltage for the better separation and resolution. This is the principle of high voltage electrophoresis.

- Voltage: Upto 10000 V and 500mA producing a potential gradient of 200V/cm.
- Time duration for separation: 10 – 60 minutes.


Insulated cover
Pressure pad
Cooling plates
Polythene sheets
– ve electrode
Buffer
Wick
Paper sheet
Cooling water
+ ve electrode

- Procedure: The electrophoresis apparatus is under total electrical insulation because of use of lethal voltage.
- It consists of two buffer reservoirs for anode and cathode and support for holding electrophoretic paper, which is connected to the buffer reservoir by means of filter paper wicks. Since high voltage is employed, lot of heat generation will occur. This may cause poor resolution.

- This heat must be dissipated by cooling plates. These cooling plates are made up of aluminium which are insulated from the suporting medium by polythene sheets.
- Cold water is circulated through the cooling plates at the rate of 10 – 15 l/minute. Before circulating the water it should be refrigerated in tropical countries. Water softener to be used in case of hard water or it will damage the cooling plates.

- Size of the cooling plates of upto 50 X 50cm is used to cover large whatmann papers. The cooling should be efficient to avoid temperature gradients because difference in 1°C will cause a change in migration velocity of 3% and affects separation.
- These cooling plates are pressed against the insulated supporting medium by an inflatable pressure pad. Electrophoresis is conducted within a time duration of 10 – 60 minutes.

- **Uses:** Separation of low molecular weight substances within a short period of time eg. protein hydrolysates, peptides, nucleic acids.
- Two dimensional electrophoresis is applied for protein hydrolysate separation with h.v.e in one direction followed by chromatography in a second dimension.

Isotachophoresis

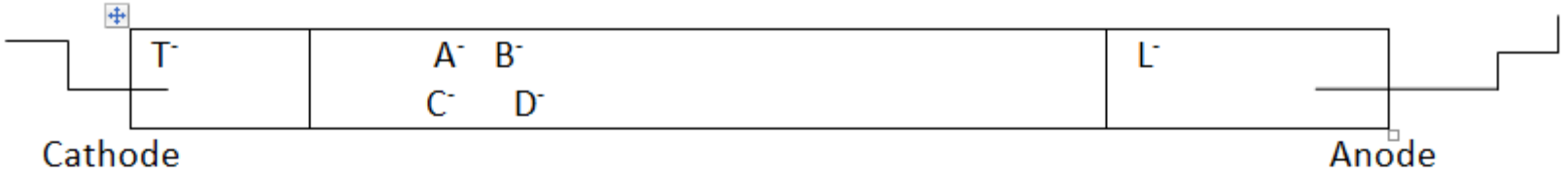
- Principle: Isotachophoresis means ions being separated all travel at the same speed. It is based on the principle of moving boundary electrophoresis where electrophoresis is conducted in a free solution without supporting medium. So the migration rate of ions are fast because of minimal frictional resistance between the solution and the sample ions.
- Molecules with the similar charge properties tend to move together as a band, and a boundary is formed between the molecules with slightly different electrophoretic mobilities. As a result the similar ionic components of the sample are stacked into discrete zones and separated in order of their migration rate. Sample is injected between leading and trailing ions.

- Time of separation: 10 – 30 minutes.
- Voltage: Upto 30KV.

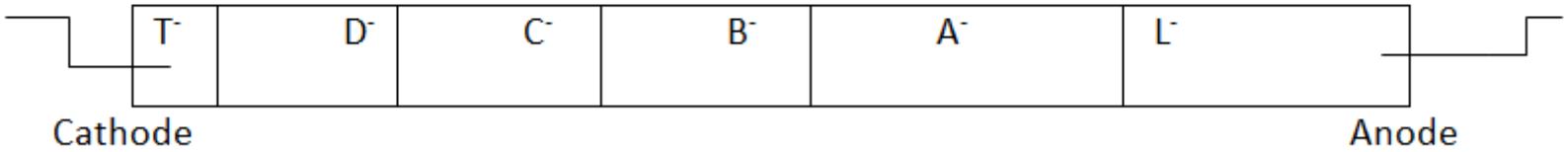
- Procedure: In this method for separating anions, leading anion, trailing anion and a common cation is required and for cations leading and trailing cation and a common anion is required.
- For the separation of anions, leading chloride anion, trailing glutamate anion and Tris as a common cation is used. The sample is injected between the leading and trailing ions.

- When the current is switched on, the leading ion will move towards the appropriate electrode followed by sample ions and finally trailing ions, when the equilibrium is reached, the ions will all move at the same speeds in the order of their mobilities as discrete bands.
- Separation is carriedout in capillary tubes which is covered by thermostatically controlled cooling bath.

- When the sample contains ions of similar mobilities separation is enhanced by the addition of ampholytes called **spacer ions** which have intermediate mobilities between sample ions and occupy the intermediate position during migration and enhance separation.
- The separated ions can be measured by measuring ultraviolet absorption.



Addition of sample mixture between leading and trailing ions



Ions migrating in the order of their mobilities at same velocity

- Uses: Separation of all charged substances from inorganic ions, organic acids, proteins and nucleic acids.
- Few micrograms to large quantities separated.
- It is used in quality control in food, industry, brewing industry and pharmaceutical industry.
- Used in pollution control by detecting detergents, inorganic ions in industrial effluents.

- Reference:
- A Biologist's Guide to Principles and Techniques of Practical Biochemistry- Eds;Bryan L William and Keith Wilson. 1981