ZONE ELECTROPHORESIS

✅ Principle:

Separates components based on their charge and size as they move through a support medium (gel, paper, or acetate membrane) under an electric field.

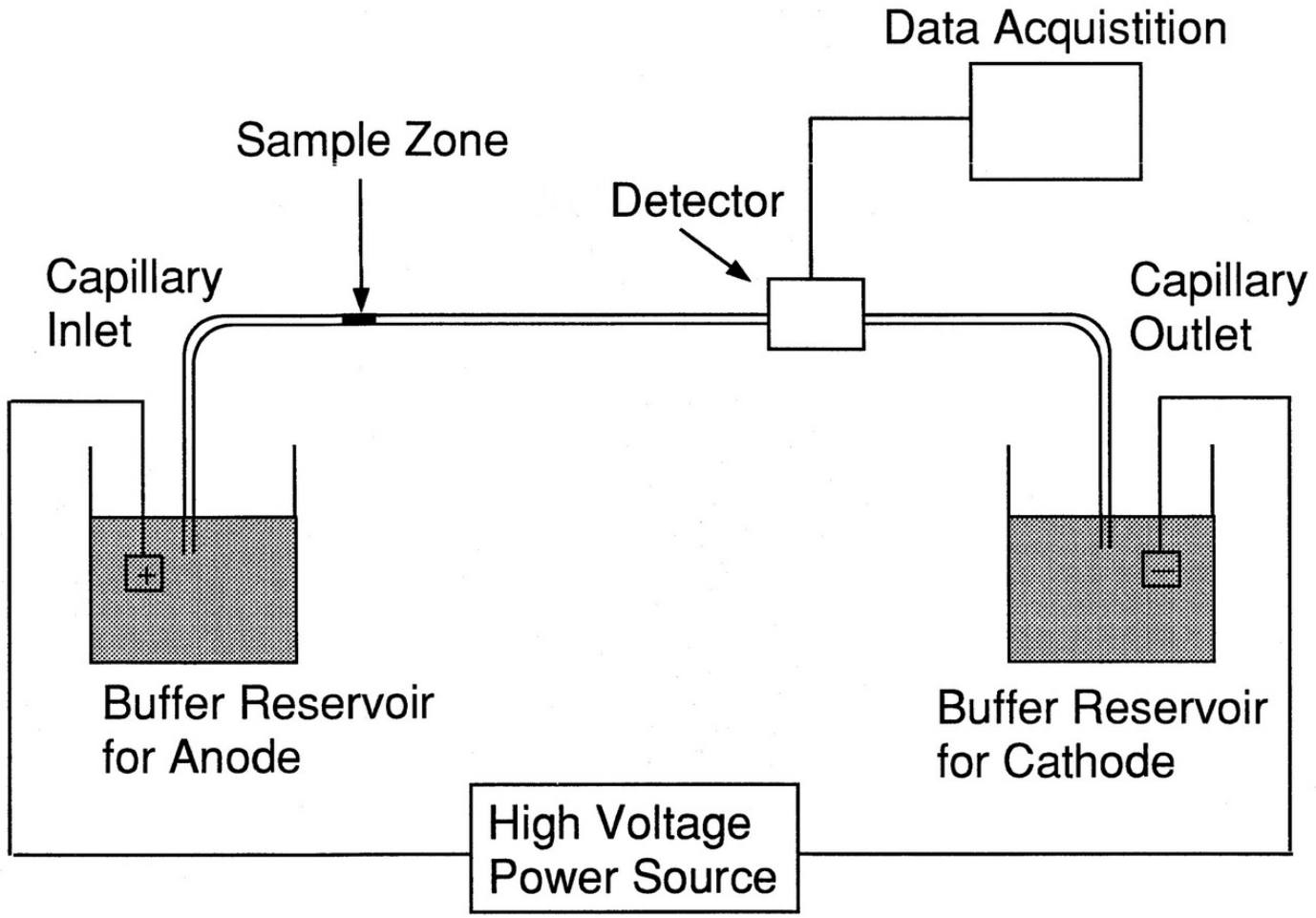
✅ Procedure:

Sample is placed in a small spot or a well in the medium.

Electric current is applied.

Molecules move at different speeds toward the anode (+) or Cathode (−) depending on their charge.

This results in forming separate zones or bands.



- Buffer Reservoirs (Anode and Cathode):

Anode (+) (with buffer) — located on the left side.
Cathode (−) (with buffer) — located on the right side.
These buffer solutions provide ions to carry the current and keep pH stable during the process.

- Capillary Tube:

This is a small, narrow tube filled with buffer solution.
The sample mixture is introduced into a small region within the tube — this forms the sample zone.

✅ High Voltage Power Source:
Provides a strong electric field across the tube.
This drives the movement of ions in the sample toward their respective electrodes — cations move toward the Cathode (−) and anions move toward the Anode (+).

✅ Detector:
Usually placed near the capillary's outlet, it detects the components as they pass by, typically by UV absorption or fluorescence.
This forms electropherograms — signals that show the different components' migration over time.

✅ Data Acquisition:
The signals are recorded by a data acquisition system (typically a computer) for further analysis.
This lets you identify and quantify components in your mixture.

MOVING BOUNDARY ELECTROPHORESIS-

✅ Principle:

Separates components in free solution under an electric field.

The boundary between the mixture components moves forward at different rates due to their different mobilities.

✅ Procedure:

Tube filled with buffer solution and mixture.

Electric field is applied.

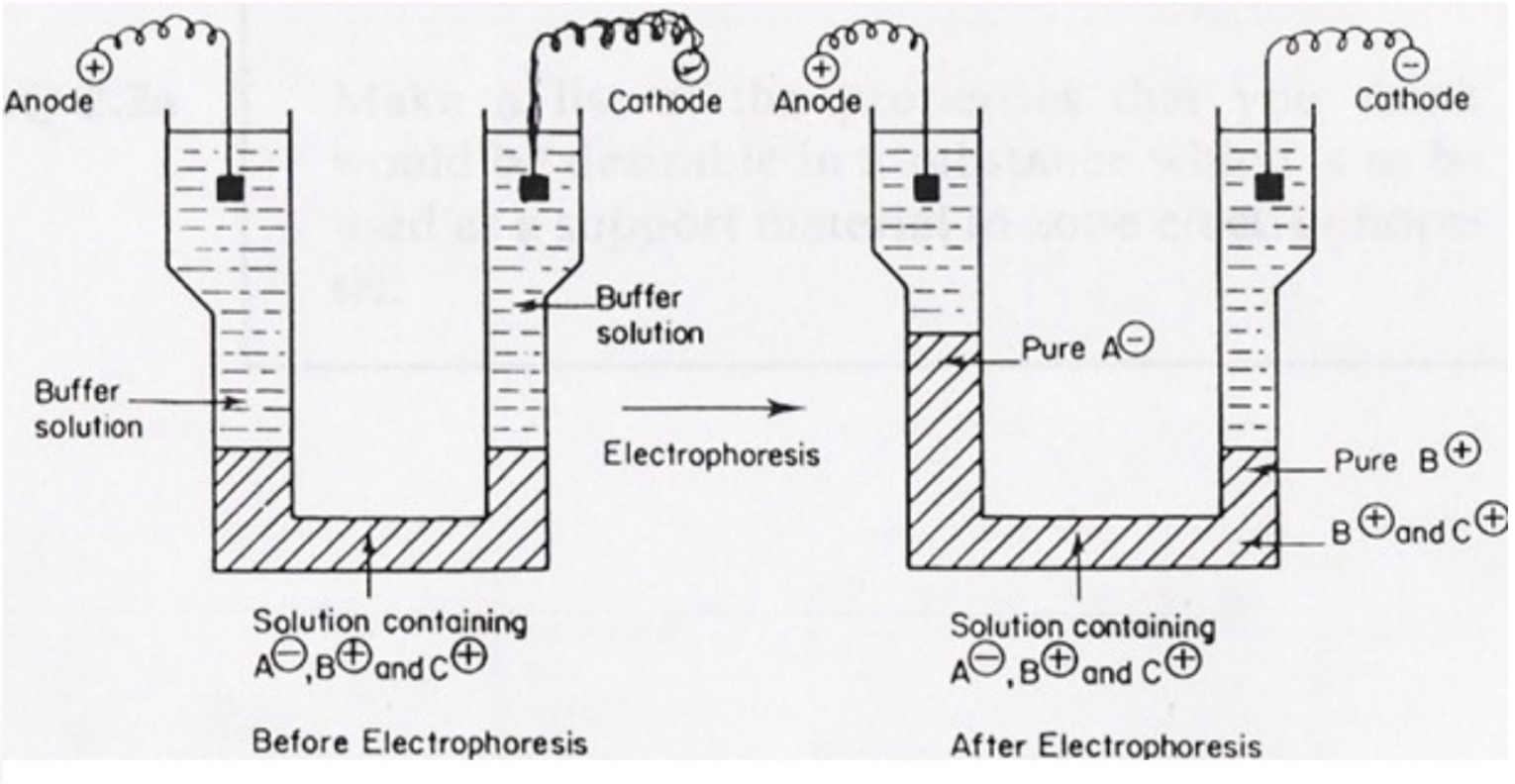
Formation of boundary between components is observed (with optical methods).

✅ Features:

Purely in solution — no support medium.

Less resolution than zone electrophoresis.

Mainly used for determining mobilities and charges of macromolecules.



1. *Support Medium-*

A piece of filter paper or gel is placed on a glass plate.

This serves as a medium through which the samples will move under an electric field.

2. *Placement of Sample:*

The sample is placed at a spot (sample origin) in the center of the medium.

3. Buffer Solutions:

Buffer solution is filled into two separate tanks — one at the anode (+) side and another at the cathode (–) side.

This buffer maintains pH and provides ions for conducting current.

4. Cloth or Paper Wick:

Paper or cloth wicks connect the buffer solution to the medium.

This forms a complete liquid path for current flow.

5. Application of Electric Field:

An electric field is applied by turning on the power supply.

The anode (+) attracts negatively charged components, while the cathode (–) attracts positively charged components.

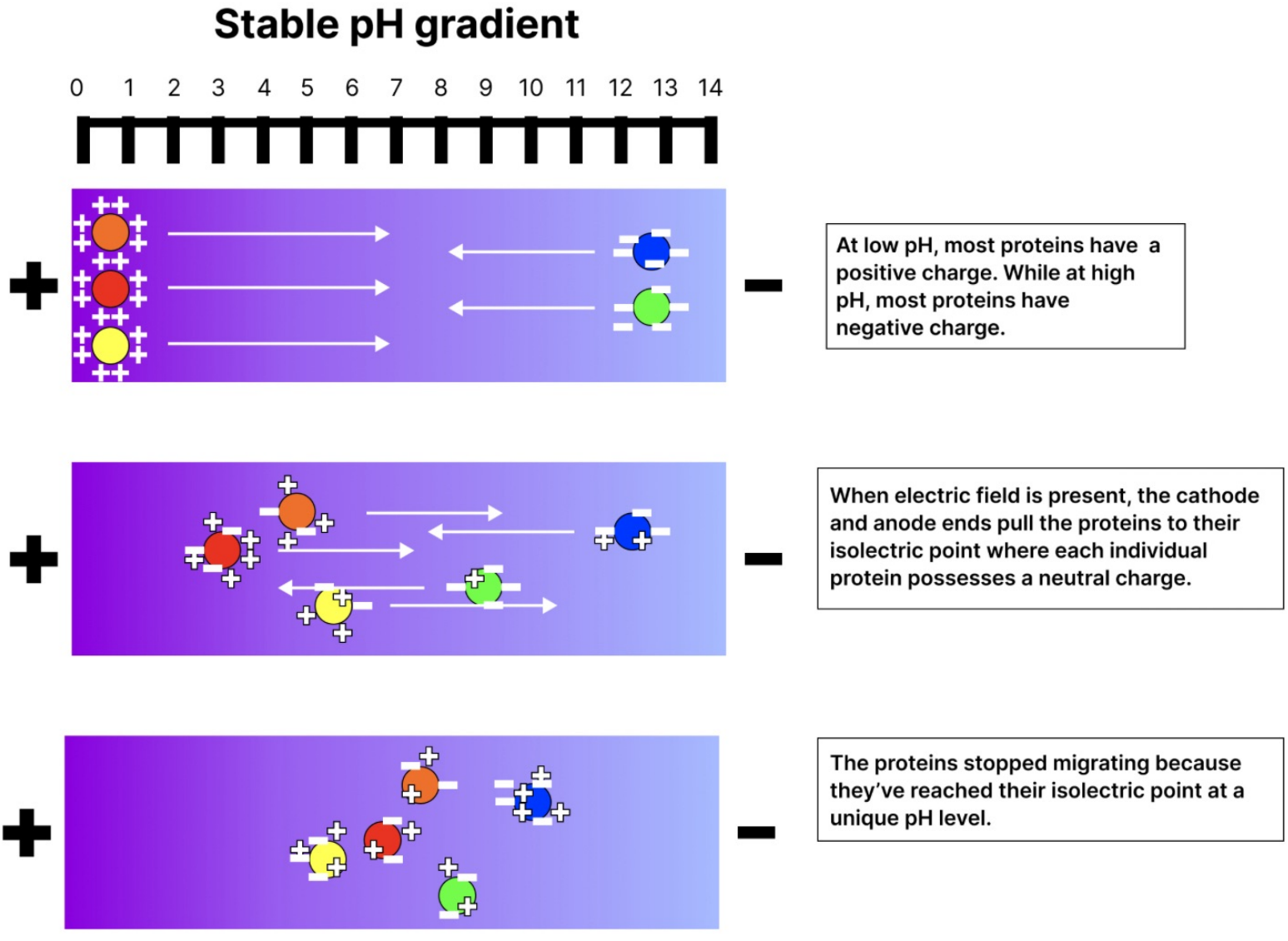
6. Migration:

The components in the sample move through the medium at different speeds depending on their charge and size.

Iso electric Focusing

Isoelectric focusing is a technique used to separate proteins based on their isoelectric point (pI) — the pH at which a protein carries no net electrical charge.

During isoelectric focusing, a pH gradient is established in a gel, and an electric field is applied. Each protein moves through the gradient until it reaches its pI, where it stops moving.



Step 1 — pH Gradient is Established -

The gel contains a stable pH gradient ranging from acidic (pH 0-1) to alkaline (pH 13-14).
At low pH, proteins carry a positive charge.
At high pH, proteins carry a negative charge.

Step 2 — Application of Electric Field

An electric field is applied across the gel.
Positively charged proteins move toward the cathode (-) side.
Negatively charged proteins move toward the anode (+) side.

Step 3 — Migration to Isoelectric Point (pI)-

Each protein moves until it reaches its isoelectric point (pI) — the pH at which it carries no net charge (neutral).
At pI:
Positive and negative charges balance.
The protein stops moving in the pH gradient.

Step 4 — Final Distribution-

Each protein forms a sharp, well-separated band at its pI.

The color-coded circles represent different proteins with their respective pI:
Orange: pI 7.5
Red: pI 6.8
Yellow: pI 5.6
Green: pI 8.5
Blue: pI 10.1