Electrophoresis

Paper Electrophoresis

Principle

Paper electrophoresis is based on the movement of charged particles under the influence of an electric field.

Basic idea:

- When an **electric field is applied**, charged molecules move toward the electrode of **opposite charge:**
  - Cations (positive ions) → move toward cathode (−)
  - Anions (negative ions) → move toward anode (+)

Medium used:

- A strip of paper soaked in buffer solution

Detailed mechanism:

- The paper holds buffer, which provides ions and maintains pH
- Sample is applied as a spot on paper
- When current is passed:
  - **Molecules move depending on:**
    - Their charge
    - Their size
    - The pH of buffer

Separation occurs because:

- Different molecules have different charge-to-mass ratios
- So, they move at different speeds

Important concept:

- At a specific pH, molecules may:
  - Be positively charged
  - Be negatively charged
  - Have no charge (will not move)

Instrumentation

1. Power supply

- Provides direct current (DC)

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2. Electrophoresis chamber

- Contains buffer reservoirs at both ends

3. Paper strip

- Acts as support medium
- Soaked in buffer

4. Electrodes

- Anode (+) and cathode (−)

5. Sample applicator

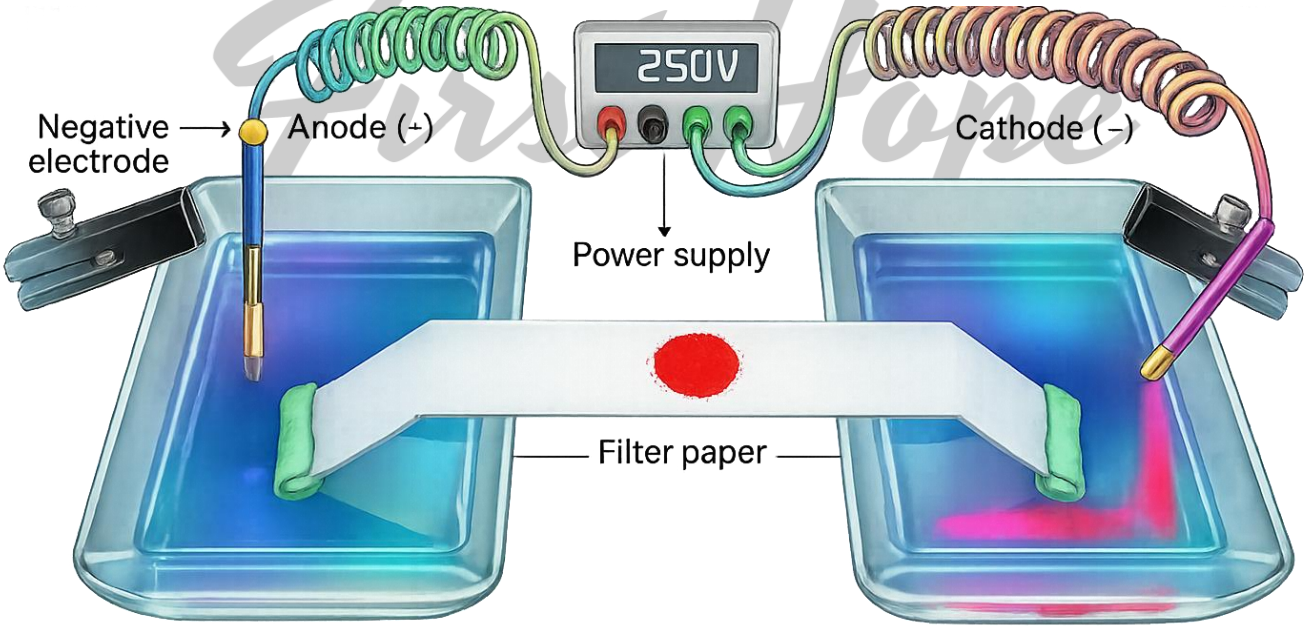
- Capillary tube or micropipette

6. Buffer solution

- Maintains pH and conductivity

7. Drying and detection setup

- Oven or air drying
- Staining reagents



Working Conditions

A. pH of buffer

- Very important
- Determines charge on molecules

B. Voltage applied

- Usually low to moderate (100–500 V)

C. Time of run

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- Depends on separation needed

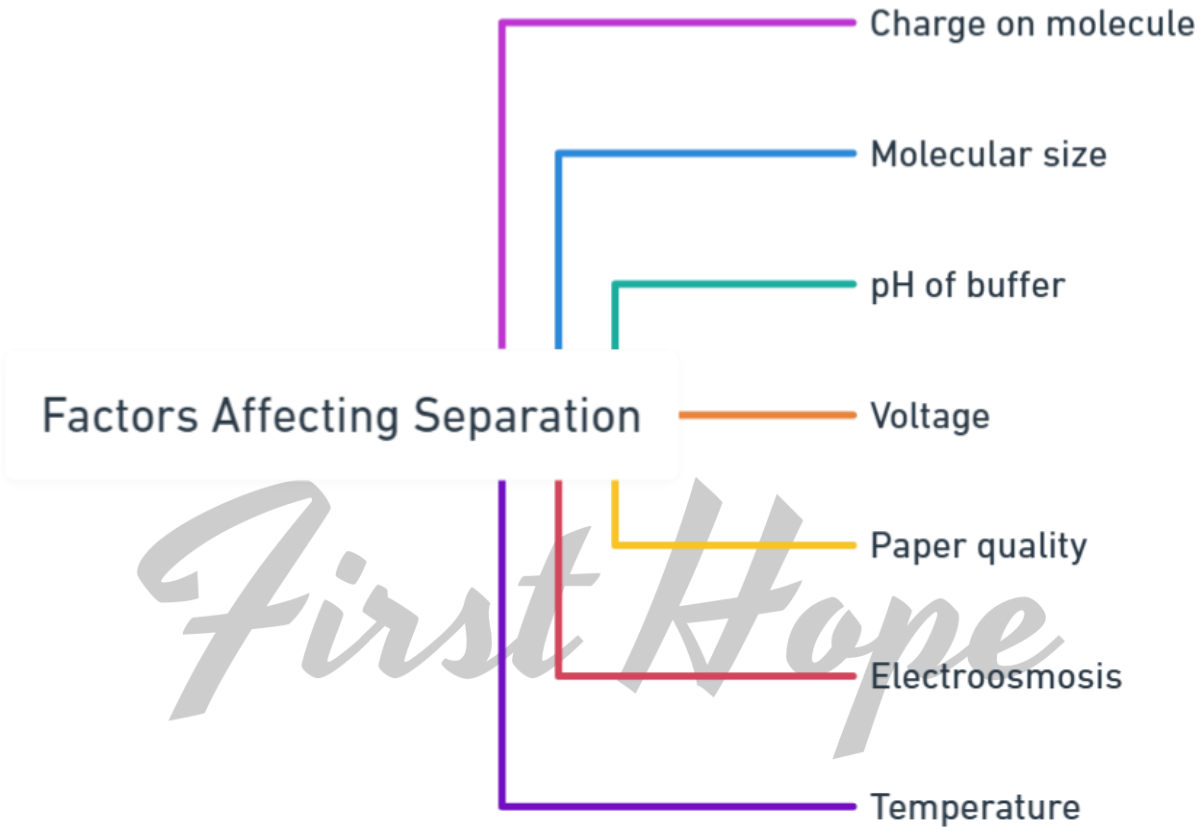
D. Temperature

- Should be controlled to avoid overheating

E. Buffer concentration

- Affects conductivity and movement

Factors Affecting Separation



1. Charge on molecule

- Higher charge → faster movement

2. Molecular size

- Smaller molecules move faster

3. pH of buffer

- Changes ionization of molecules

4. Voltage

- Higher voltage → faster movement but may cause diffusion

5. Paper quality

- Uniformity affects migration

6. Electroosmosis

- Movement of liquid affects separation

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7. Temperature

- High temperature can distort results

Applications

1. Separation of amino acids
2. Analysis of proteins
3. Study of nucleotides
4. Clinical diagnosis (serum proteins)
5. Identification of charged biomolecules

Gel Electrophoresis

Principle

Gel electrophoresis is based on movement of charged molecules through a gel under an electric field, with separation mainly based on size and charge.

Medium used:

- Gel (agarose or polyacrylamide)

Basic idea:

- Molecules move through gel pores when electric field is applied

Detailed mechanism:

- Gel acts like a **molecular sieve**
- Smaller molecules pass easily → move faster
- Larger molecules face resistance → move slower

Separation occurs because:

- **Differences in:**
  - Size
  - Shape
  - Charge

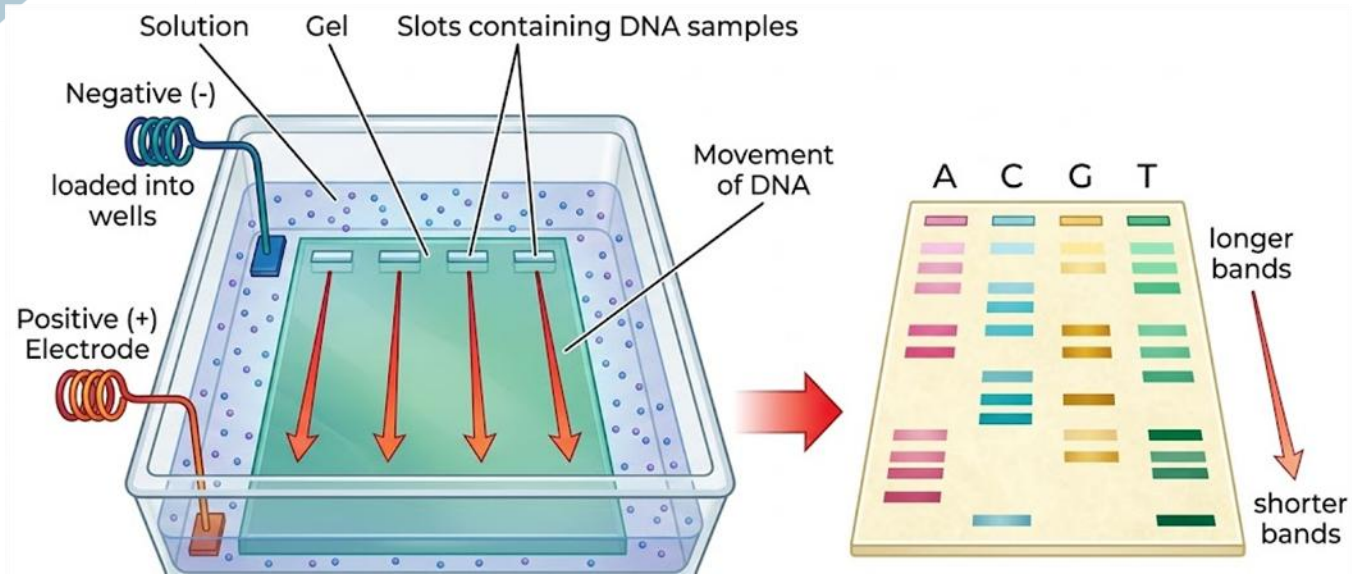
Important concept:

- DNA is negatively charged → moves toward anode
- Proteins can be separated based on size (using SDS-PAGE)

Instrumentation

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1. **Power supply**
   - Provides DC current
2. **Gel casting tray**
   - Used to prepare gel
3. **Gel (stationary medium)**
   - Agarose (for DNA)
   - Polyacrylamide (for proteins)
4. **Electrophoresis tank**
   - Contains buffer and gel
5. **Electrodes**
   - Anode and cathode
6. **Sample wells**
   - Formed in gel for loading samples
7. **Buffer solution**
   - Maintains pH and conductivity
8. **Staining system**
   - Ethidium bromide or other dyes
9. **UV transilluminator**
   - For visualization

Working Conditions

A. Voltage

- Typically 50–200 V

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