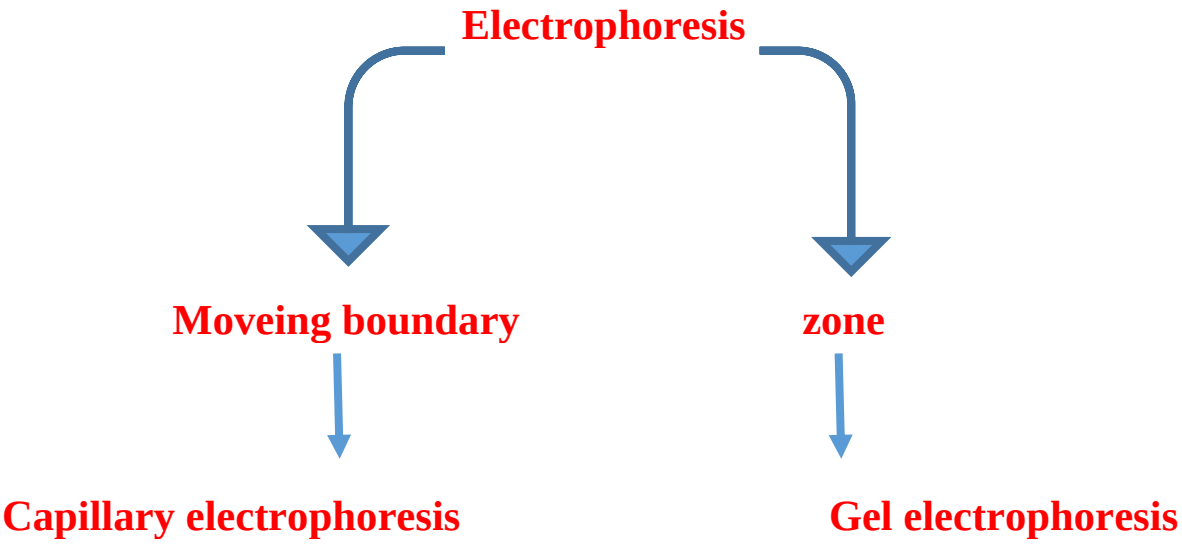
Name of student: …… **Stage: First**

Lecture Name: Gel electrophoresis and water bath **Number: 6**

Date: 28 / 4 / 2025 **Time: 8:30 AM** **Place: BC 208**

Electrophoresis: is a laboratory technique used to separate molecules like DNA, RNA, or proteins based on their **size** and **charge** by applying an electric field.



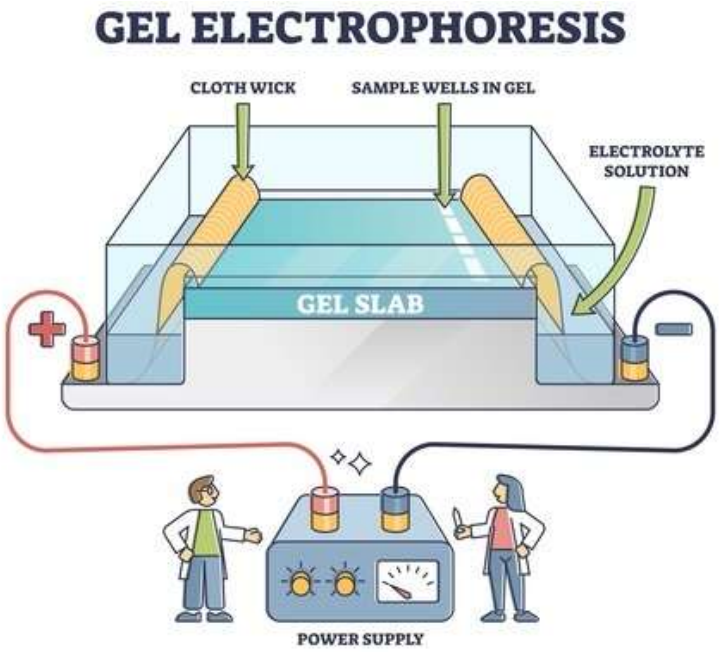
Gel electrophoresis:

is a laboratory technique used to separate and analyse DNA, RNA, and protein molecules based on their size, charge, and other physical properties. It involves placing the sample to be analysed on a porous gel matrix, applying an electric field, and then allowing the molecules to migrate through the gel matrix.

The molecules are separated based on their size, with smaller molecules moving faster through the gel than larger ones.

The technique was first developed in the 1940s and 1950s by biochemists Arne Tiselius and Archer Martin. Since then, gel electrophoresis has become an essential tool in molecular **biology** and **biochemistry** used to analyse and purify a wide range of biomolecules.

Gel electrophoresis is a widely used analytical technique in molecular biology and biochemistry It is a simple and effective way to isolate and purify specific molecules from complex mixtures, such as DNA fragments, RNA transcripts, or proteins. Gel electrophoresis is used in a variety of applications, including genetic research, medical diagnostics, and biotechnology.



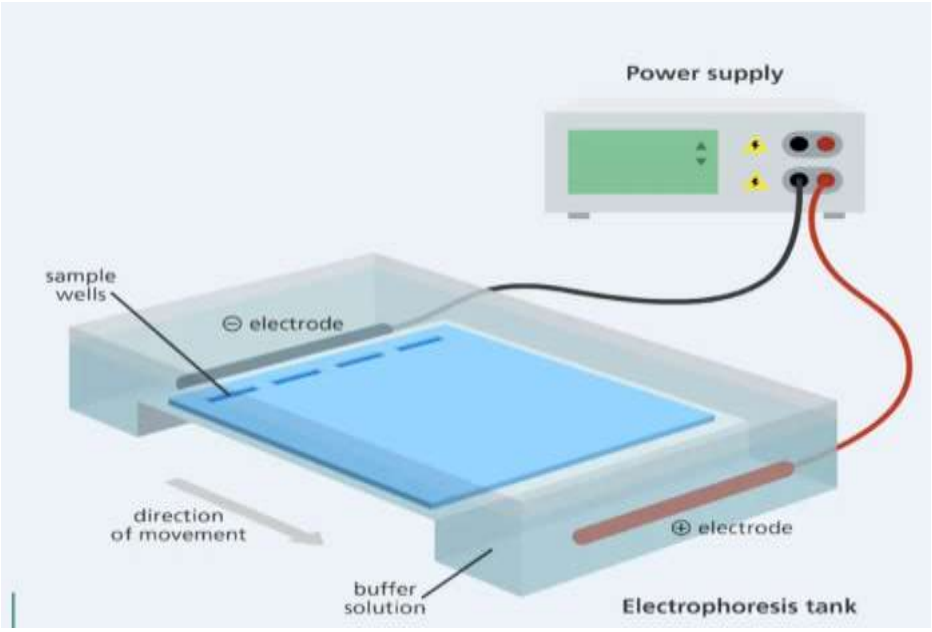
Principle Gel electrophoresis:

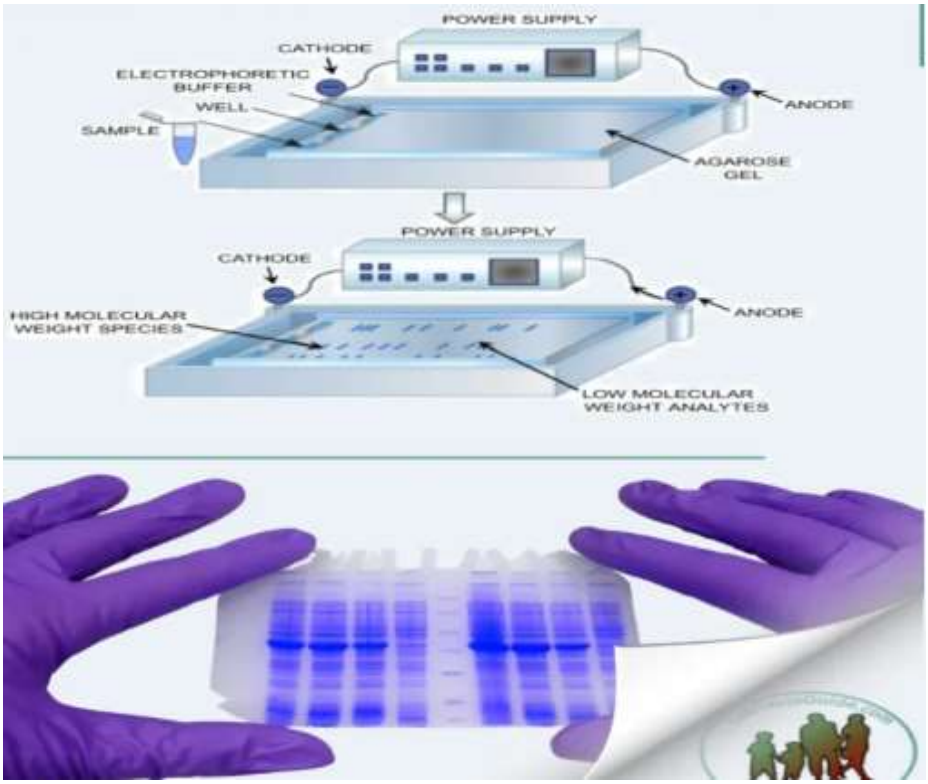
- DNA, RNA, and proteins are negatively charged and move towards the positive electrode in an electric field.
- Gel matrix: Made of agarose for (DNA) or polyacrylamide (for proteins and small RNA).
- Function of the gel: Acts as a porous medium that separates molecules based on size.
- Sample preparation: Mixed with loading buffer containing dye and denaturing agents.
- Loading and running: Samples are loaded into wells; electric current is applied.
- Migration: Smaller molecules move faster and farther through the gel.

- Visualization: Separated molecules are stained (e.g., ethidium bromide for DNA).
- Further analysis: Molecules can be extracted from the gel for additional experiments.

Requirements and instruments for agarose gel electrophoresis:

- **Electrophoresis chamber:** to run the electric current through the gel.
- **Power supply:** to provide the required voltage for DNA migration.
- **Gel casting tray:** to shape and hold the agarose gel; usually UV-transparent plastic.
- **Sample combs:** to create wells in the gel for loading samples.
- **Agarose powder:** to prepare the gel matrix.
- **Electrophoresis buffer:** typically TAE (Tris-acetate-EDTA) or TBE (Tris-borate-EDTA).
- **Microwave or heating device:** to dissolve agarose in buffer.
- **Loading buffer:** contains glycerol (for weight) and tracking dyes.
- **DNA samples:** the nucleic acids to be separated.
- **Staining agent:** commonly ethidium bromide or other DNA dyes (e.g., SYBR Safe).
- **Transilluminator (UV light box)**: to visualize DNA bands after staining.
- **Gloves and safety gear:** for safe handling of chemicals and UV exposure.





Applications of Agarose Gel Electrophoresis

1. DNA Fragment Analysis:

Used to separate and visualize DNA fragments by size, often after restriction enzyme digestion.

2. PCR Product Verification:

Confirms the presence, size, and purity of PCR amplification products.

3. RNA Transcript Analysis

Gel electrophoresis can also be used to separate and analyses RNA transcripts of varying sizes. This is particularly useful in gene expression studies, where scientists can use gel electrophoresis to analyse the levels of different RNA transcripts in cells or tissues.

4. Protein Analysis:

Gel electrophoresis can be used to separate and analyse proteins based on their size and charge. This is particularly useful in protein purification and characterization, where scientists can use gel electrophoresis to isolate and purify specific proteins from complex mixtures.

5. DNA Fingerprinting:

Common in forensic investigations and paternity testing to compare DNA samples.

6. Medical Diagnostics:

Gel electrophoresis is also used in medical diagnostics, particularly in the diagnosis of genetic disorders. For example, gel electrophoresis can be used to analyses the hemoglobin molecules in blood samples, allowing doctors to diagnose hemoglobinopathies such as sickle cell anemia.

water bath

Definition the water bath: A water bath is laboratory instrument. It is a container or vessel filled with heated water. The temperature of water is maintained at a constant level. It is used to incubate samples over a period of time at a constant temperature.

The main parts of water bath:

1-Container or tank bath

2-Heater

3-Thermometer

4-Thermostat or regulator.

Types of water bath:

1-Circulating water bath

2-Non-circulating water bath.

3-Shaking water bath

Circulating water bath: is also called stirrer water bath. It is perfect for uses when temperature uniformity and regularity are critical; water is thoroughly distributed throughout the bath resulting in a uniform temperature: It is used for some specific substances or chemical reaction and reagents for example, enzymatic and serologic experimentations. To ensure an optimum temperature uniformity throughout the whole bath. An electric motor with rotary magnet is flanged to the bath bottom

Non-circulating water bath: It is less accurate in terms of temperature control. This type of water bath depend on mainly on convection instead of water being uniformly heated.

Shaking water bath: This type of water bath has additional control for shaking , which moves liquids from place to another place. Shaking feature can be turned on or turned off. Constant shaking allows the incubated liquid sample to be mix in constant level and the temperature is maintained at a constant point.

Principle of water bath:

principle of water bath This device depends on the heat applied to the sample using the heater.

Operating the Water Bath:

1. Connect the power supply.
2. Ensure the water level in water bath is sufficient to pour the heating element.
3. Switch “ON” the main power supply and instrument mains.
4. For temperature settings, Press SET key to set the required temperature. press ↑ to increase the temperature and ↓ to reduce the temperature
5. The temp. Sensor will maintain the set temp. During use of water bath.
6. Switch “OFF” the instrument mains & main power supply after use.

Uses of water bath:

1-used to improve the solubility of poorly soluble substances.

2-It used for melting of some substances.

3-It used for warming of chemical reagents.

4- It used for facilitating of some chemical reactions.

5. For incubation of cell cultures.

6. It is used as a heat source for some substance such as flammable chemicals.

Practical application:

1-If the equipment has been stored in cold or humid conditions, condensation may form inside it. Therefore, allow time (at least 2 hours) for the condensation to evaporate before using the equipment.

2-It is not recommended to use water bath with moisture sensitive reactions.

3-Water level should be regularly monitored and filled with distilled water or deionized water. This is required to prevent salts from depositing on the heater.

4-Disinfectants or bactericidal agents can be added to prevent growth of organisms.

5-For the purpose of decontamination the temperature of water bath may be raise to 90°C or higher to once a week for half an hour.

6-If application involves liquids that give out vapors (gases), It is recommended to operate water bath in gas hood or in a well ventilated area.

7-The cover is closed to prevent evaporation and to help reaching high temperatures.

8-Set up on a steady surface away from flammable materials.

9-Change the water regularly and empty when not in use for prolonged periods.
10- Before emptying a bath, allow the water temperature to fall to a safe level
11- Do not use the equipment in an area where there are aggressive or explosive chemical mixtures.

12- Do not use the bath to heat any material that could cause a fire or any other kind of hazard.

Water bath instrument