Al-Mustaqbal University

College of Science

Biochemical Techniques

2nd Lecture

Electrophoresis

Second Year Students / 2nd semester / 2024-2025

By

Prof. Dr. Naser Abdulhasan Naser

ELECTROPHORESIS

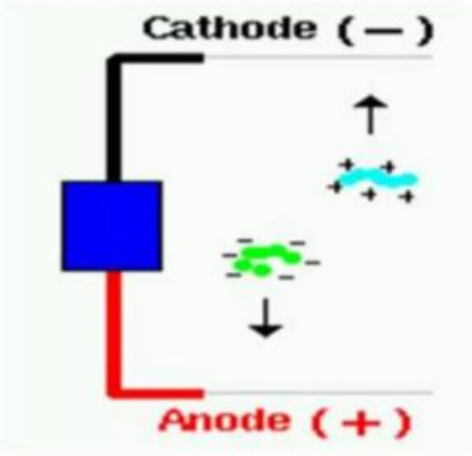
Greek word meaning **"transport by electricity"**
(*Electro* – electricity, *phoresis* – movement)

Electrophoresis is a physical analysis which involves the **separation** of the compounds that are capable of acquiring **electric charge** in conducting electrodes.

DEFINITION

- Electrophoresis may be defined as the **migration of the charged particle** through a solution under the influence of an external **electrical field.**

- Ions that are suspended between the two electrodes tends to travel towards the electrodes that bears **opposite charge**.



PRINCIPLE

Any charged ion or molecule migrates when placed in an electric field, the rate of migration depend upon its **net charge, size, shape** and the **applied electric current**.

$$v=Eq/F$$

Where,

v = velocity of the molecule

E = electric field (Volt/cm)

q = net charge on molecule

F = frictional coefficient, which depends upon mass and shape of the molecule.

The rate of migration of an ion in an electrical field depends on,

1) Net charge of molecule
2) Size, mass and shape of the particle
3) Strength of electrical field
4) Properties of supporting medium
5) Temperature of operation

FACTORS AFFECTING ELECTROPHORESIS

1) SAMPLE

a) **Charge**: rate of migration increases with increases in net charge

b) **Size**: rate of migration decreases for larger molecules. It is due to frictional and electrostatic forces.

c) **Shape**: molecules have similar charge but differ in shape exhibit different migration rate

2) ELECTRIC FIELD

a) **Voltage:** increase in voltage leads to increase in rate of migration

b) **Current:** increase in current leads to increase in voltage, migration also increases

c) **Resistance:** resistance increases and migration decreases.

3) BUFFER

a) **Ionic strength:** rate of migration increases in high ionic strength

b) **pH:** ionization of organic acid increases as pH increases

4) SUPPORTING MEDIUM

a) **Adsorption:** reduces both rate of migration and resolution of separation of molecule

b) **Electro-endosmosis:** it is due to the presence of charged groups on the surface of the supporting medium.

Ex: paper(carboxyl group), agarose (sulphate group) .

It will accelerate the movement of cations, but retard the anion movements.

TYPES OF ELECTROPHORESIS

a) ZONE ELECTROPHORESIS:

1) Paper electrophoresis
2) Gel electrophoresis
3) Thin layer electrophoresis
4) Cellulose acetate electrophoresis

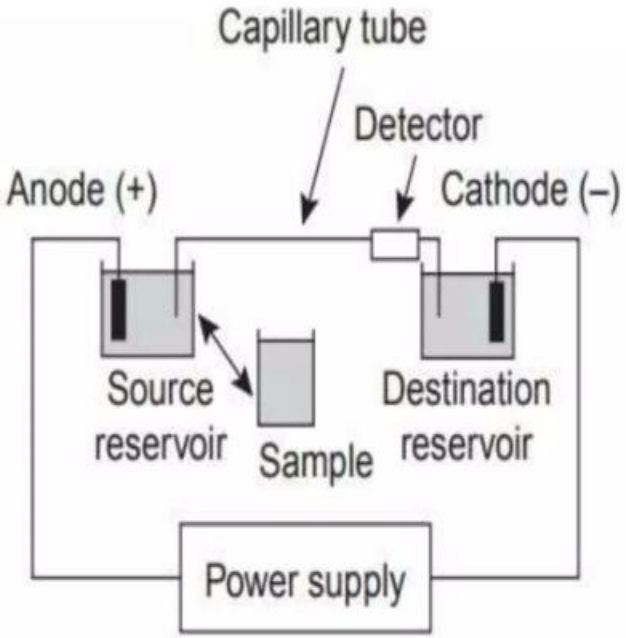
b) MOVING BOUNDARY/FRONTAL ELECTROPHORESIS:

1) Capillary electrophoresis
2) Isotachophoresis
3) Isoelectric focussing
4) Immuno-electrophoresis

INSTRUMENTATION
(General Apparatus)

It consists basically of items,

1) **Power pack**
2) **Electrophoresis unit**
   - Electrodes
   - Buffer reservoir
   - Support for electrolysis medium
   - A transparent insulating cover
3) **Sample injector**
4) **Detector**



GENERAL METHOD OF OPERATION

Saturation of the medium with the buffer

↓

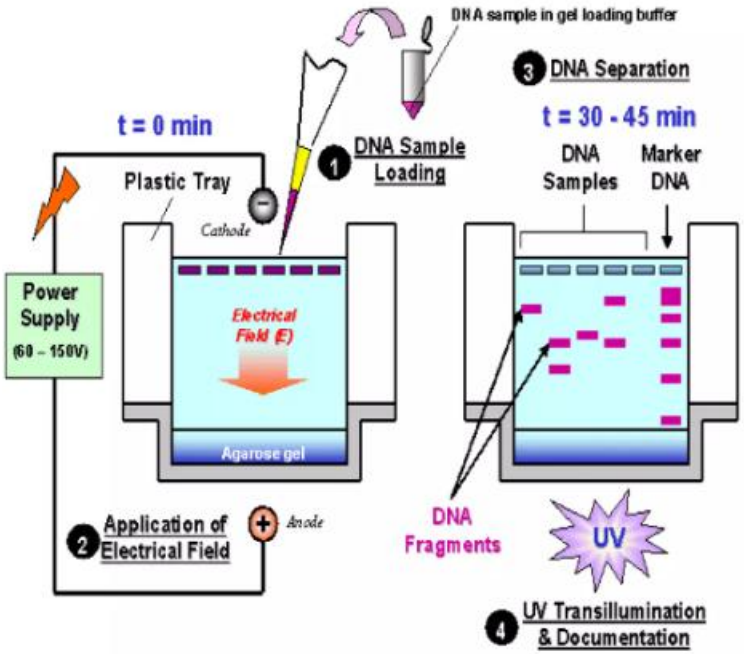
Sample application

↓

Electrophoretic separation

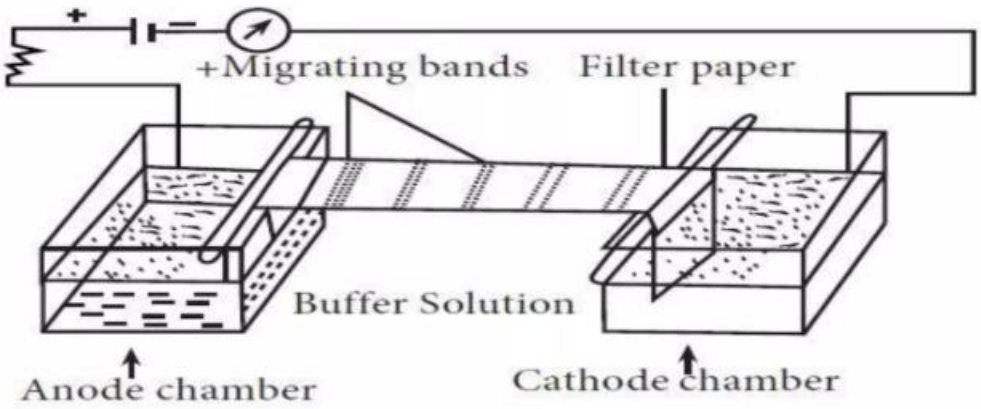
↓

Detection



1) PAPER ELECTROPHORESIS

It is the form of electrophoresis that is carried out on **filter paper**. This technique is useful for separation of **small charged molecules** such as amino acids and small proteins.



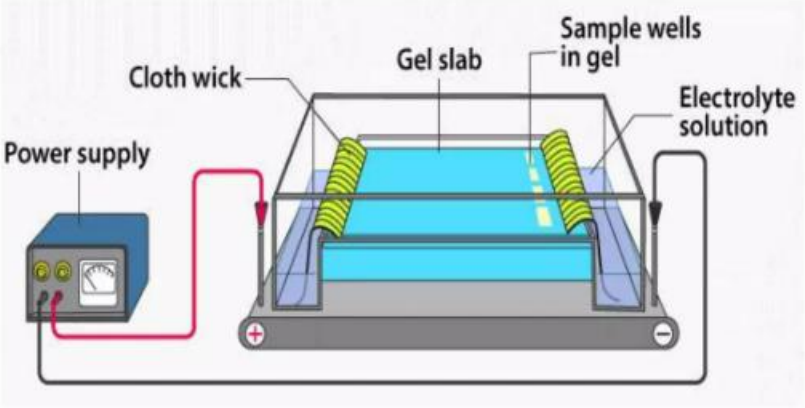
2) GEL ELECTROPHORESIS

It is a technique used for the separation of **DNA, RNA or protein** molecules according to their **size** and **electrical charge** using an electric current applied to a gel matrix.

GEL: Gel is a **cross linked polymer** whose composition and porosity is chosen based on the **specific weight** and **porosity** of the target molecules.

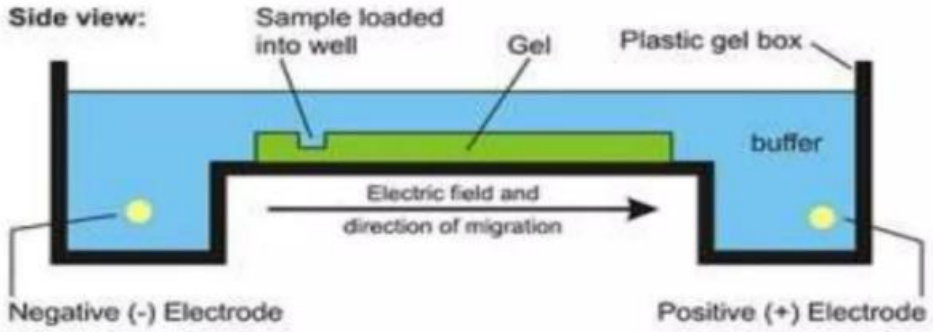
TYPES OF GEL

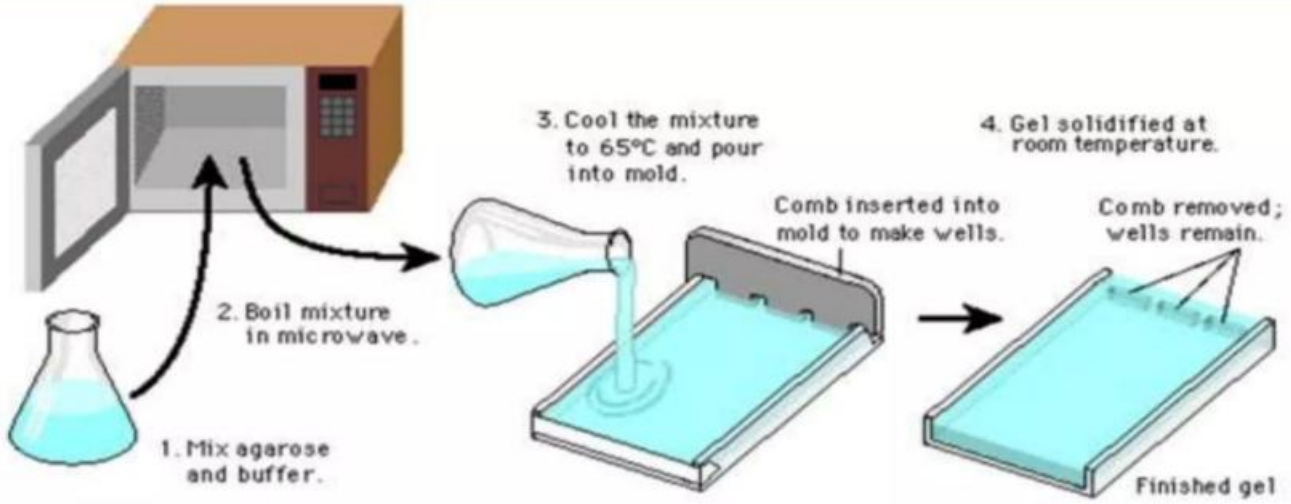
1) Agarose gel
2) Polyacrylamide gel
3) Sephadex gel



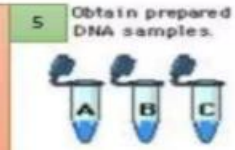
AGAROSE GEL ELECTROPHORESIS

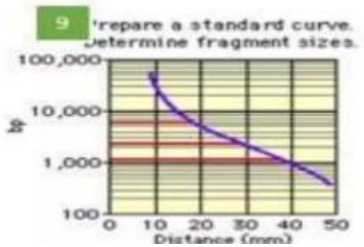
- A highly purified uncharged polysaccharide derived form **agar**.
- Used to separate macromolecules such as **nucleic acid, larger proteins and protein complexes**.
- It is prepared by dissolving **0.5% agarose** in boiling water and allowing it to cool to **40°C**.
- It is **fragile** because of the formation of weak hydrogen bonds and hydrophobic bonds.

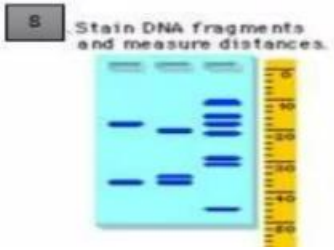


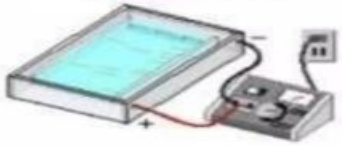


An overview of gel electrophoresis









SDS PAGE ELECTROPHORESIS

- **S**odium **D**odecyl **S**ulphate **P**oly-**A**crylamide **G**el **E**lectrophoresis
- SDS is an **anionic detergent** which binds strongly to and denature proteins.
- Protein SDS complex carries net **negative charges**, hence move towards anode and the separation is based on the **size** of the particle and also be used for determining the relative **molecular mass** of a protein.

APPLICATIONS:

1) Useful for **protein purification**.
2) Determine molecular weight of proteins.
3) Quantifying proteins
4) Blotting applications

b) MOVING BOUNDARY ELECTROPHORESIS

PRINCIPLE:

The moving boundary method allows the charged species to migrate in a **free moving solution** without the supporting medium.

ADVANTAGES:

1) Biologically active fractions can be recovered without the use of denaturing agents.
2) Minute concentrations of the sample can be detected.

DISADVANTAGES:

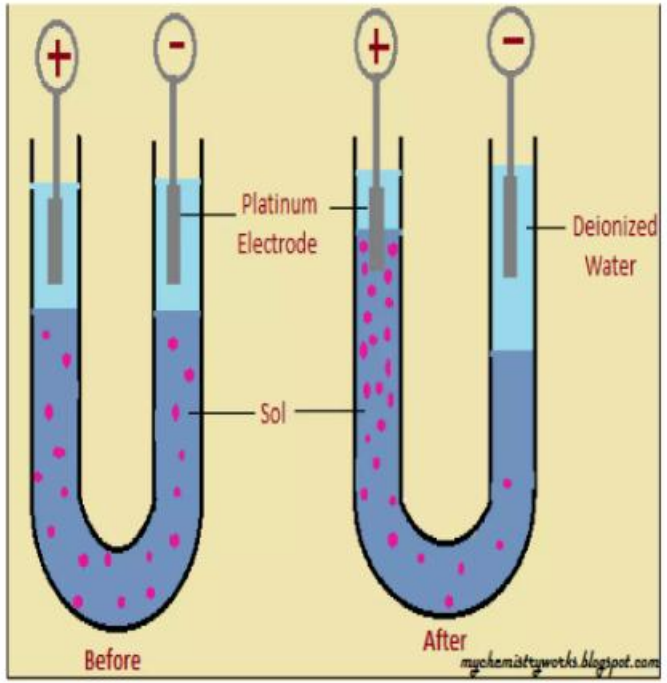
1) Costlier
2) Elaborate optical system are required.

APPLICATIONS:

1) To study homogenecity of a macromolecular system.
2) Analysis of complex biological mixture.

INSTRUMENTATION

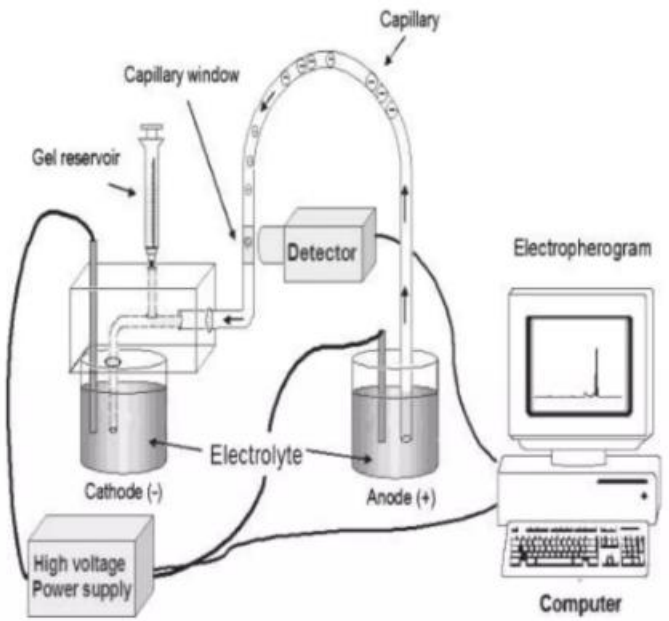
- Consists of a **U shaped** glass cell of rectangular cross section with electrodes placed on the one each of the limbs of the cell.
- Sample solution is introduced at the bottom or through the side arm and the apparatus is placed in a constant temp. bath at 40°C.
- Detection is done by measuring **refractive index** throughout the solution (Schileren optical system).

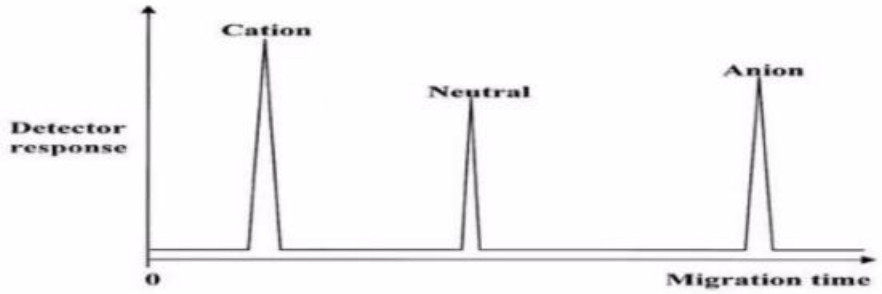


1) CAPILLARY ELECTROPHORESIS

- Capillary tube is placed between the two buffer reservoir and an electric field is applied, separation depends on **electrophoretic mobility** and **electro-osmosis**.
- Defined volume of analyte is introduced into the capillary by replacing one buffer reservoir with sample vial.
- Electrophoretic separation is measured by detector.
- Using narrow bore tubes, capillary electrophoresis removes the joule heating effect which decreases band broadening, giving faster separations than gel.
- CE uses tubes **20-100µm diameter** and **20-100cm length**.
- CE is used with/without gel.
- Longitudinal diffusion is the main source of band-broadening.

- Higher electric fields result in **high efficiency** and **narrow peaks** (analyte migrates faster).
- All analytes travel the same distance, but the **migration time (tm)** for that distance is measured.
- Relate time to identity.
- Relate peak area or height to amount.





2) ISOTACHOPHORESIS

- ***Iso*** – equal, ***tachos*** – speed, ***phoresis*** – migration
- The technique of isotachophoresis depends on the development of **potential gradient**.
- Used for separation of **smaller ionic substances**.
- They migrate adjacent with contact one another, but not overlapping.
- The sample is **not mixed with buffer** prior to run.
- Hence current flow is carried entirely by the sample ions.

APPLICATION:

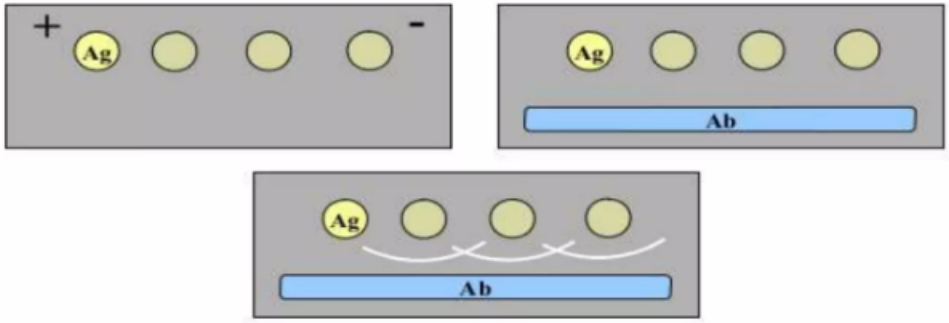
Separation of small anions and cations.

4) IMMUNO ELECTROPHORESIS

- When electrical field is applied to study **of antigen-antibody reactions**, it is called immuno-electrophoresis.
- The antibody are electrophoretically separated and antigens diffuse towards each other resulting in **precipitin arcs** where antigen antibody complexes form.
- It is used to detect the **presence of antibodies**.
- Used mainly to determine **the blood levels** of three major **immunoglobulins** :
  - **IgM**
  - **IgG**
  - **IgA**

PROCESS

- Ag molecules are separated according to differences in their **electrical charge** by electrophoresis.
- Antibody is placed in a trough cut in the agar.
- Antibody diffuse towards antigen → **precipitin arcs**.
- **Interpretation** – precipitin arc represent individual antigens.

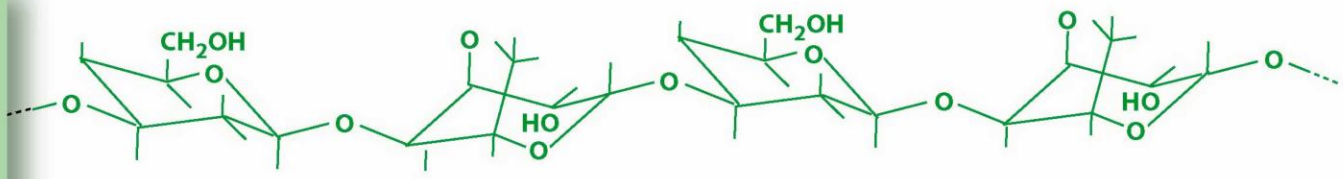


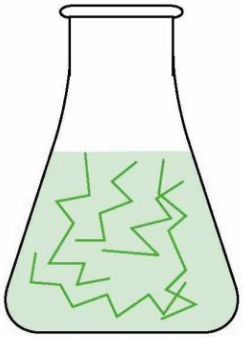
APPLICATIONS OF ELECTROPHORESIS

1) DNA sequencing
2) Medical research
3) Protein research/purification
4) Agricultural testing
5) Separation of organic acid, alkaloids, carbohydrates, amino acids, alcohols, phenols, nucleic acids, insulin.
6) In food industry
7) It is employed in biochemical and clinical fields ie., in the study of protein mixtures such as blood serum, haemoglobin and in the study of antigen-antibody interactions.
8) Electrophoresis in combination with auto-radiograhy is used to study the binding of iron to serum proteins.

Agarose Gel

Agarose is extracted from seaweed, and is a linear polymer of sugar molecules.





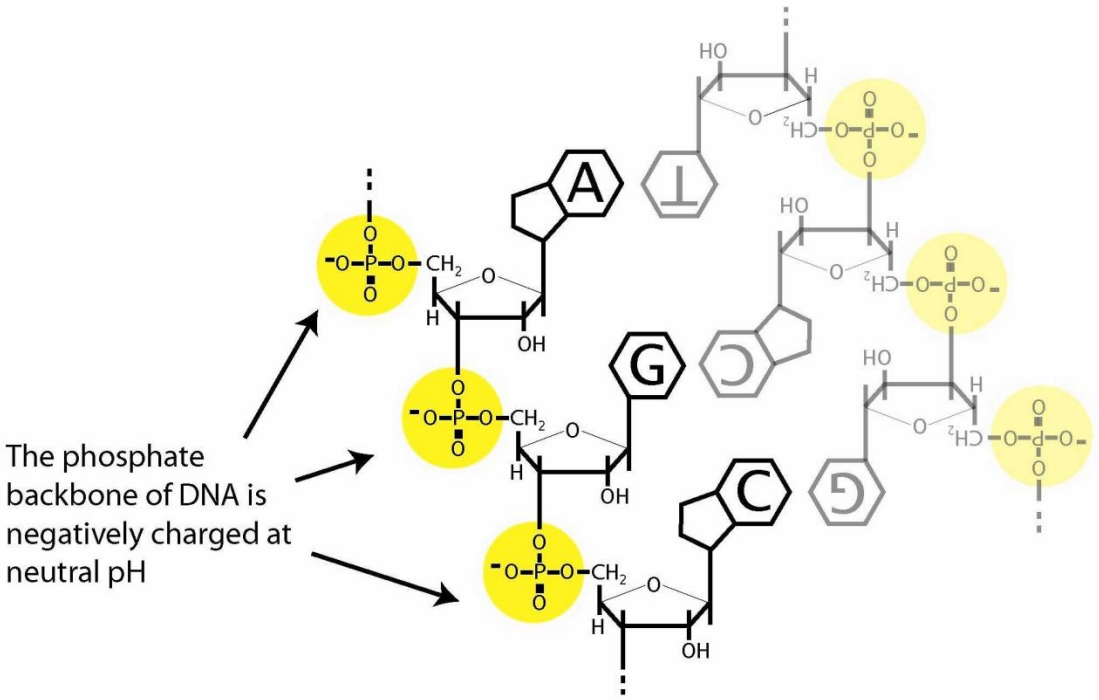
When heated in water, the agarose polymers are flexible and the mixture of agarose and water liquid.

When the agarose and water cool, the agarose polymers form a matrix. When this happens, the mixture becomes more solid and gel-like. The concentration of agarose determines how dense the matrix is.

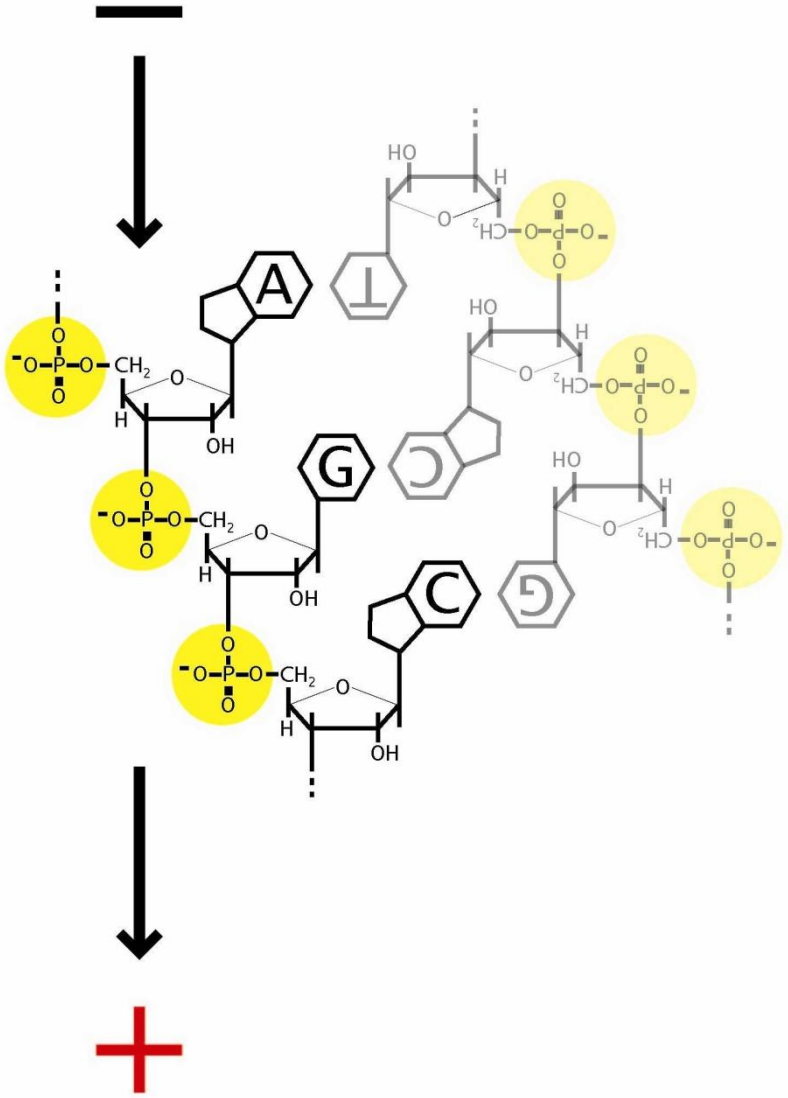
Lower concentration of agarose

Higher concentration of agarose

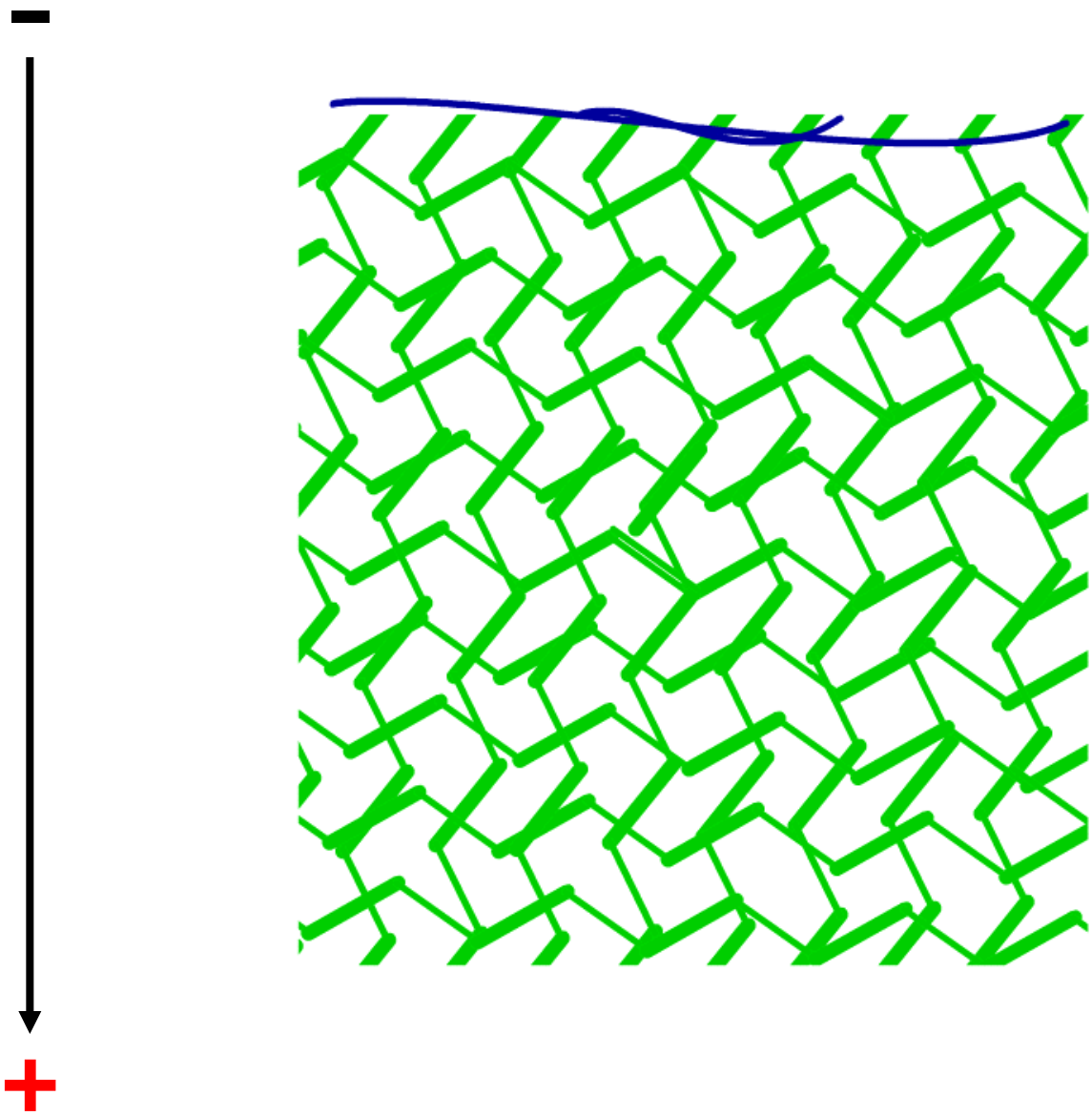
DNA

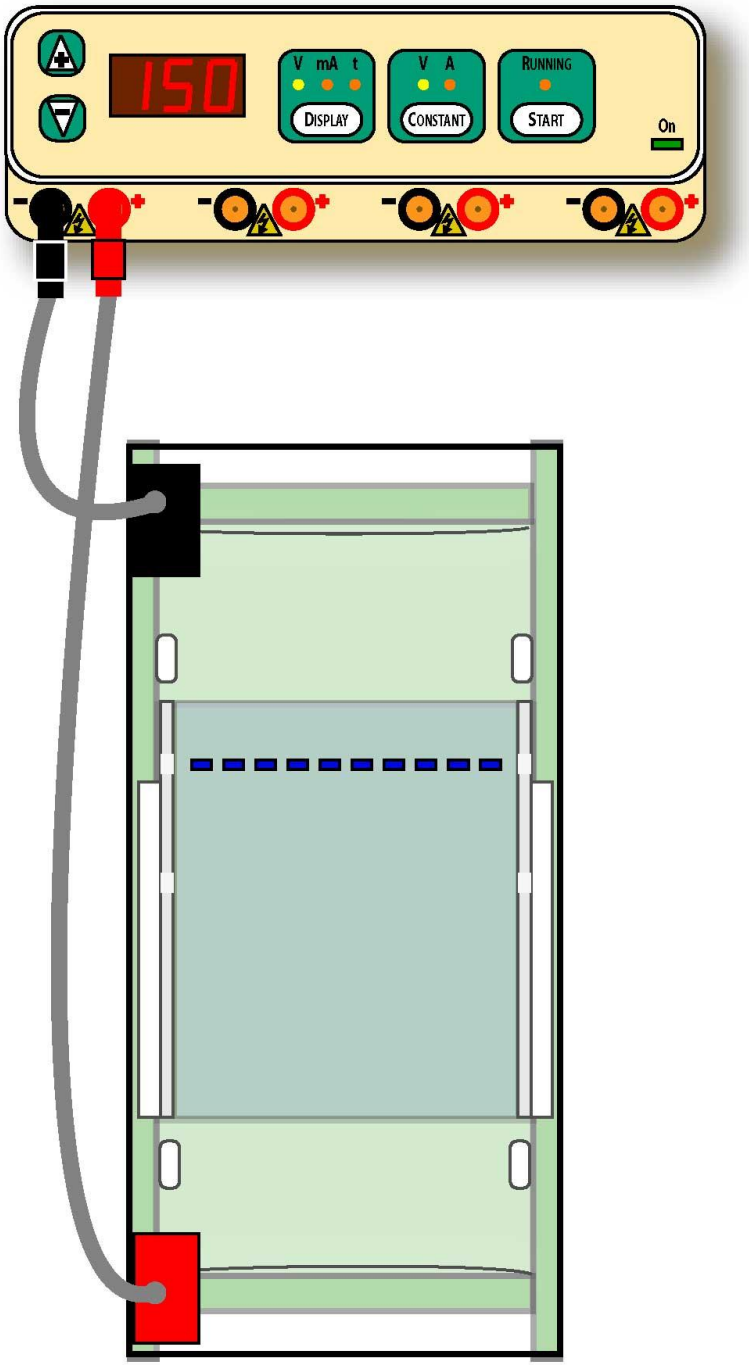

The phosphate backbone of DNA is negatively charged at neutral pH
A
G
C
T
HO
H
OH
O
P
CH2

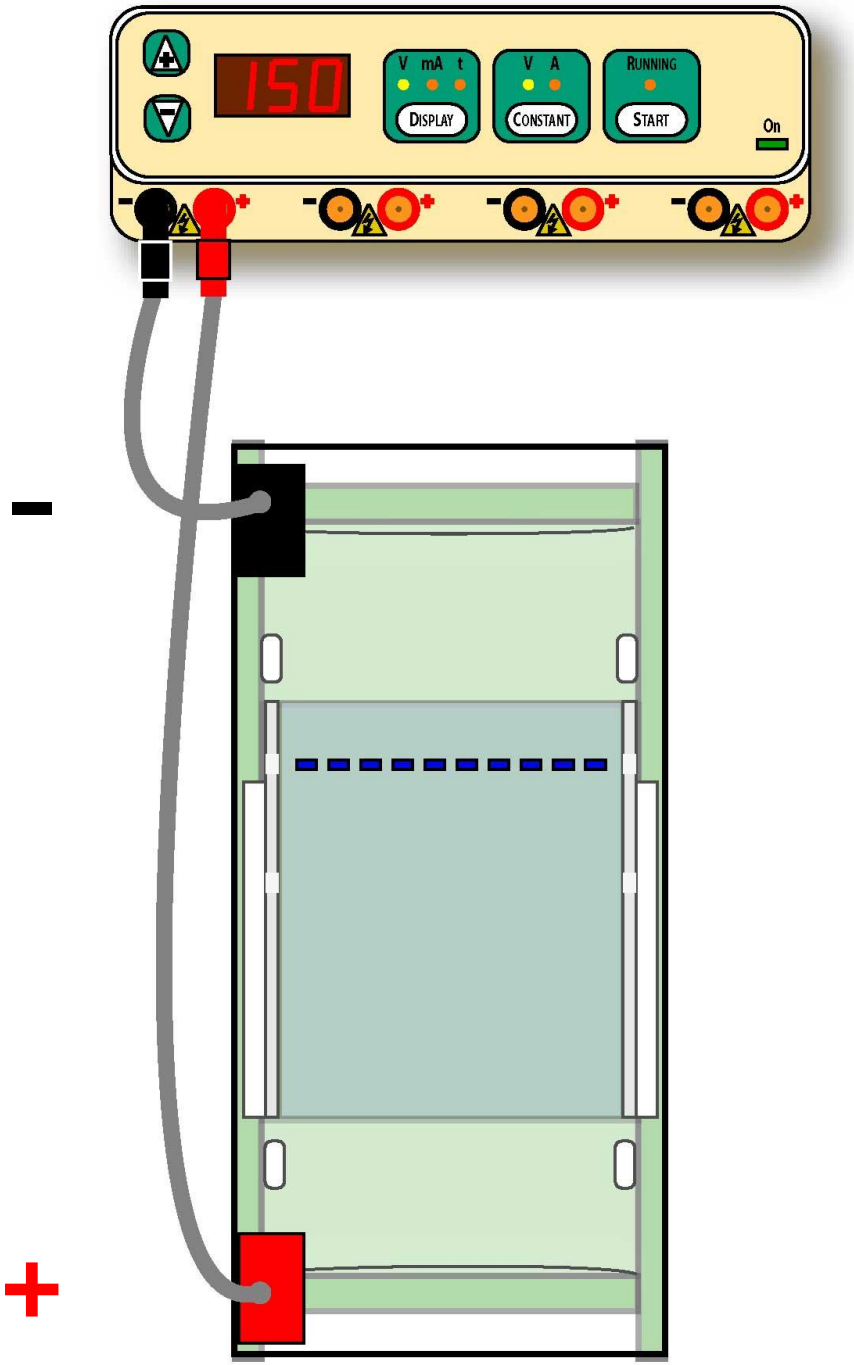
DNA and Electrical Current



DNA and Electrical Current




150
V mA t
DISPLAY
V A
CONSTANT
RUNNING
START
On


150
V mA t
DISPLAY
V A
CONSTANT
RUNNING
START
On
-
+

DNA and Electrical Current

Many DNA fragments of the same size migrate together on the gel as a “band”

https://www.youtube.com/watch?v=QRt-8ChlbT4