Electrophoresis

By

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Introduction

- When a potential difference is applied between two electrodes in a colloidal solution, It has been observed that the colloidal particles are carried to either the positive or negative electrode.

. In other words , they behave as if they are electrically charged particele move in the dispersion medium.

This phenomenon is known as ***electrophoresis.***

- The movement of charged particle in an electric field is expressed in terms of electrophoretic mobility.

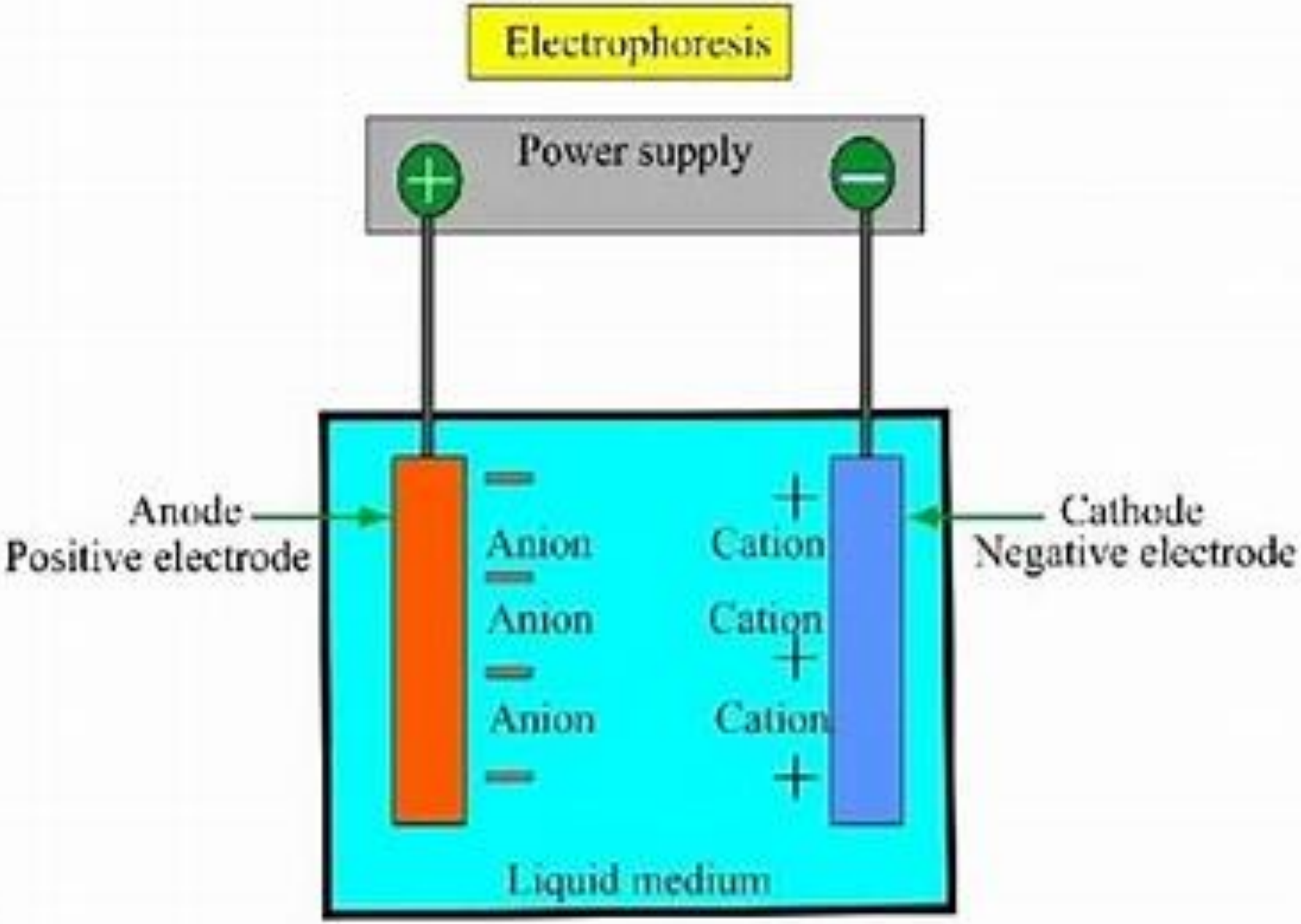
- Electrophoresis is a simple and sensitive separation technique in clinical and research laboratories. Since its discovery, it has been an essential tool used by biologists and chemists to separate mixtures, especially proteins and nucleic acids.
- Electrophoresis consists of two words; electro, meaning electricity, and phoresis, meaning movement. Thus, it implies the migration and separation of a charged particle (ions) through a solution under the influence of an electric field.

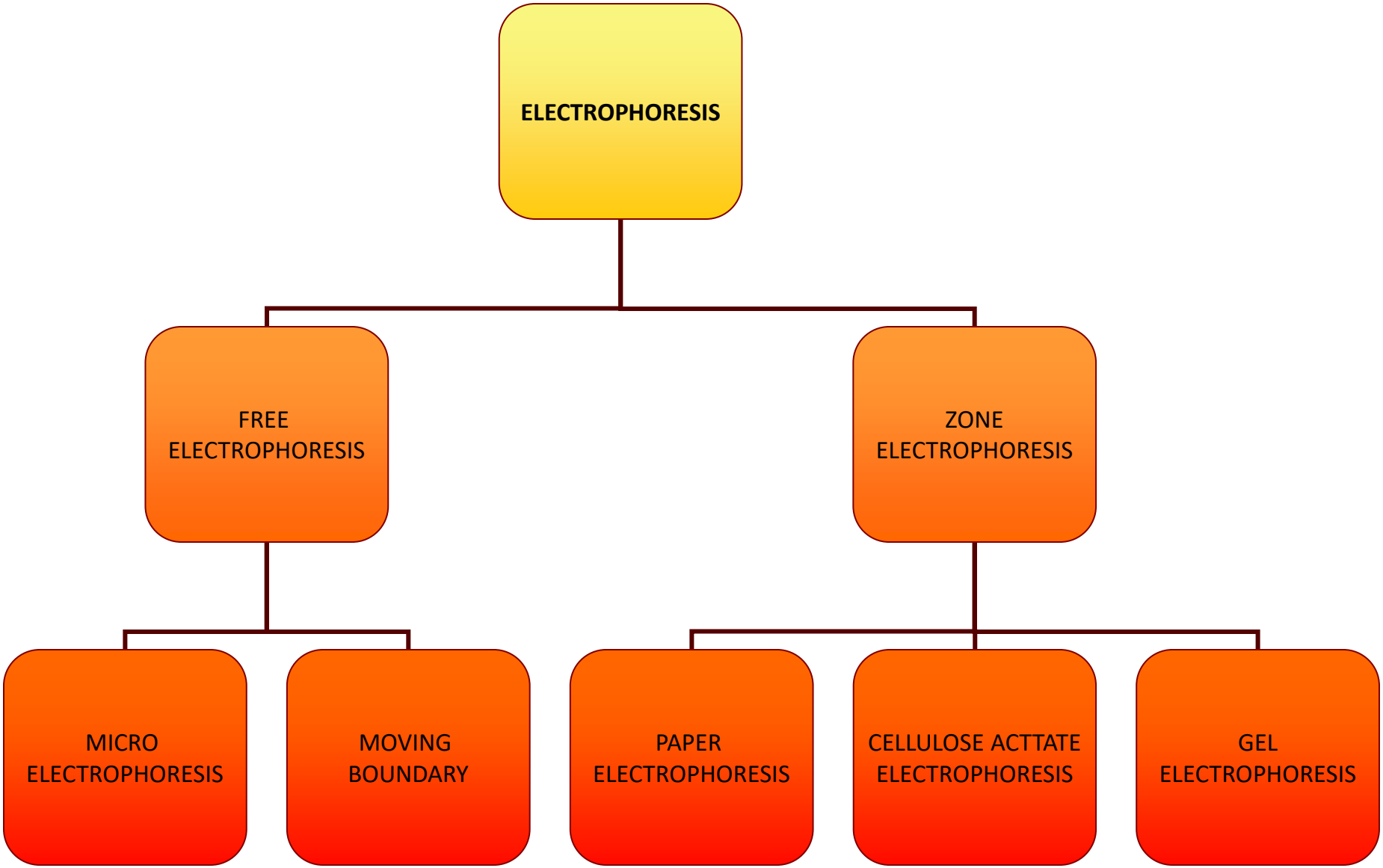
- Many important biological molecules, such as amino acids, peptides, proteins, nucleotides and nucleic acids, possess ionisable groups and, therefore, at any given pH, exist in solution as electrically charged species either as cations or anions.

- Under the influence of an electric field these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge.

Electrophoresis

- Electrophoresis is defined as the “migration of charged molecules under the influence of an external electric field”
- Electrophoresis is a technique used for the separation of biological molecules based on their movement due to the influence of a direct electric current.
- The technique was pioneered in 1937 by the Swedish chemist **Arne Tiselius** for the separation of proteins.
- It has now been extended to the separation of many other different classes of biomolecules including nucleic acids, carbohydrates and amino acids.


Electrophoresis
Power supply
Anode
Positive electrode
Cathode
Negative electrode
Anion
Anion
Anion
Cation
Cation
Cation
Liquid medium


ELECTROPHORESIS
FREE ELECTROPHORESIS
ZONE ELECTROPHORESIS
MICRO ELECTROPHORESIS
MOVING BOUNDARY
PAPER ELECTROPHORESIS
CELLULOSE ACTTATE ELECTROPHORESIS
GEL ELECTROPHORESIS

PRINCIPLE

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

Can be represented by following eq.

$$v = \frac{E * q}{f}$$

- v = velocity of migration of the molecule.
- E = electric field in volts per cm
- q = net electric charge on the molecule
- f = frictional coefficient

- Biological molecules, like amino acids, peptides, proteins, nucleic acids, and nucleotides, possess ionizable groups. These molecules exist in solution as electrically charged species, cations (+), or anions (-) at any given pH.
- Thus, the electric field allows the migration of the negatively charged molecule towards the anode (a positive terminal). In contrast, the positively charged molecule migrates towards the cathode (a negative terminal).
- The separation of the molecules, ions, or colloidal particles suspended in the matrix occurs due to the force of an electric field. The molecules move through a sieve-like compound based on the molecular mass and charge ratio.

- yet the molecules separate due to electrophoretic mobilities.
- Nucleic acids have negative phosphate backbones. Hence they move towards the anode in DNA electrophoresis.
- Ampholytes, like proteins, bear both positive and negative charges. Such compounds have negative charge in normal conditions and migrate towards the anode. At the same time, they are positively charged in acidic conditions and move towards the cathode. Hence, protein bears a negative or positive charge depending on solvent pH and isoelectric point.

- **Factors Affecting the Rate of Ion Mobility**
- The velocity of ions depends on both inherent factors and the external environment.
- **Inherent factors**
- The inherent factors that affect the velocity of ions are:
- Charge density
- Molecular weight
- The net charge of the molecule

- **External factors**
- The external factors affecting the rate of movement of ions are:
- Electrical parameters, like current, voltage, and power
- Viscosity and pore size of supporting medium
- Temperature
- The pH of the buffer

- **Electrophoresis Instrument**
- Modern electrophoresis equipment and systems vary based on its types and forms. However, all the electrophoretic system possesses two essential components:
- **Power pack**
- Power supply drives the movement of ionic species in the medium and allows adjustment and control of either the current or the voltage.
- **An electrophoresis unit**
- An electrophoretic system depends on its type but essentially consists of two electrodes of opposite charge (anode and cathode), connected by a conducting medium called an electrolyte.
- In addition, a supportive medium is present in electrophoretic systems like gel and paper electrophoresis.

- **Buffer (Electrolyte)**
- Buffers carry applied electric current and provide appropriate pH for the process. Conducting (running) buffers like Tris borate EDTA (TBE) and Tris-acetate acid EDTA (TAE) are commonly used.
- **Supportive Medium**
- The supportive medium is the matrix (gel), in which biomolecules are separated. It can be in the slab or capillary form. The supportive mediums used are sugar polymers like agarose gel, polyacrylamide gel, starch gel, and cellulose acetate gel. The medium runs either vertical or horizontal gel systems in gel electrophoresis. Horizontal: agarose gel electrophoresis, and vertical: SDS-PAGE. The higher the pore size, the higher the speed traveled by charged particles.

General Procedure of Electrophoresis

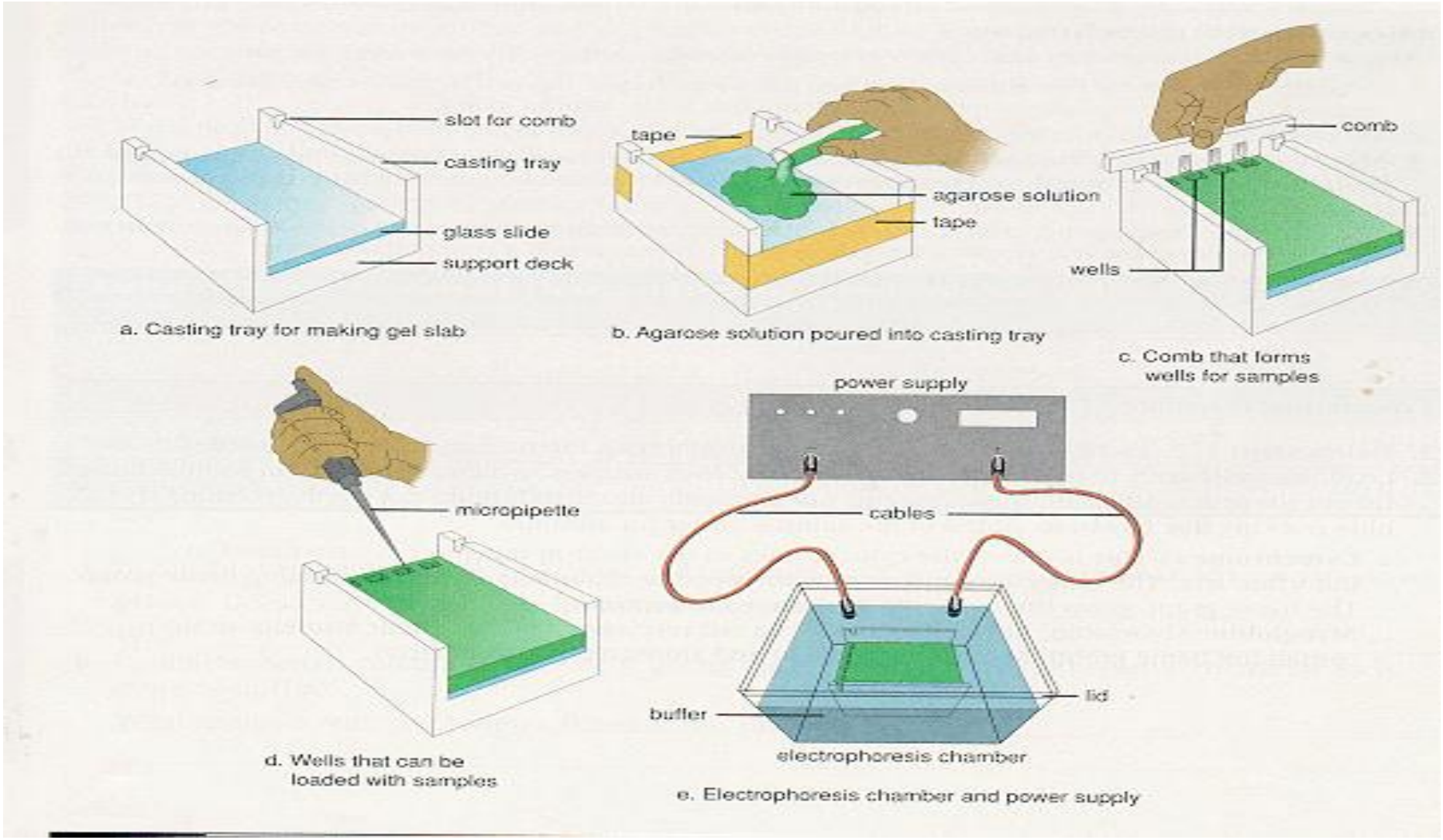
- The electrophoresis process has three main steps; **separation, detection, and quantification.**
- **Separation**
- The instrument set up is according to its type. In the gel electrophoresis, gels are prepared and cast. Then placed into the electrophoresis chamber.
- The supportive medium can be agarose gels or polyacrylamide gels.
- Then appropriate buffer solution is added to the system.
- After the proper setup of the instrument, the sample is placed into the medium. Then the sample is run at a specific current, voltage, or power.

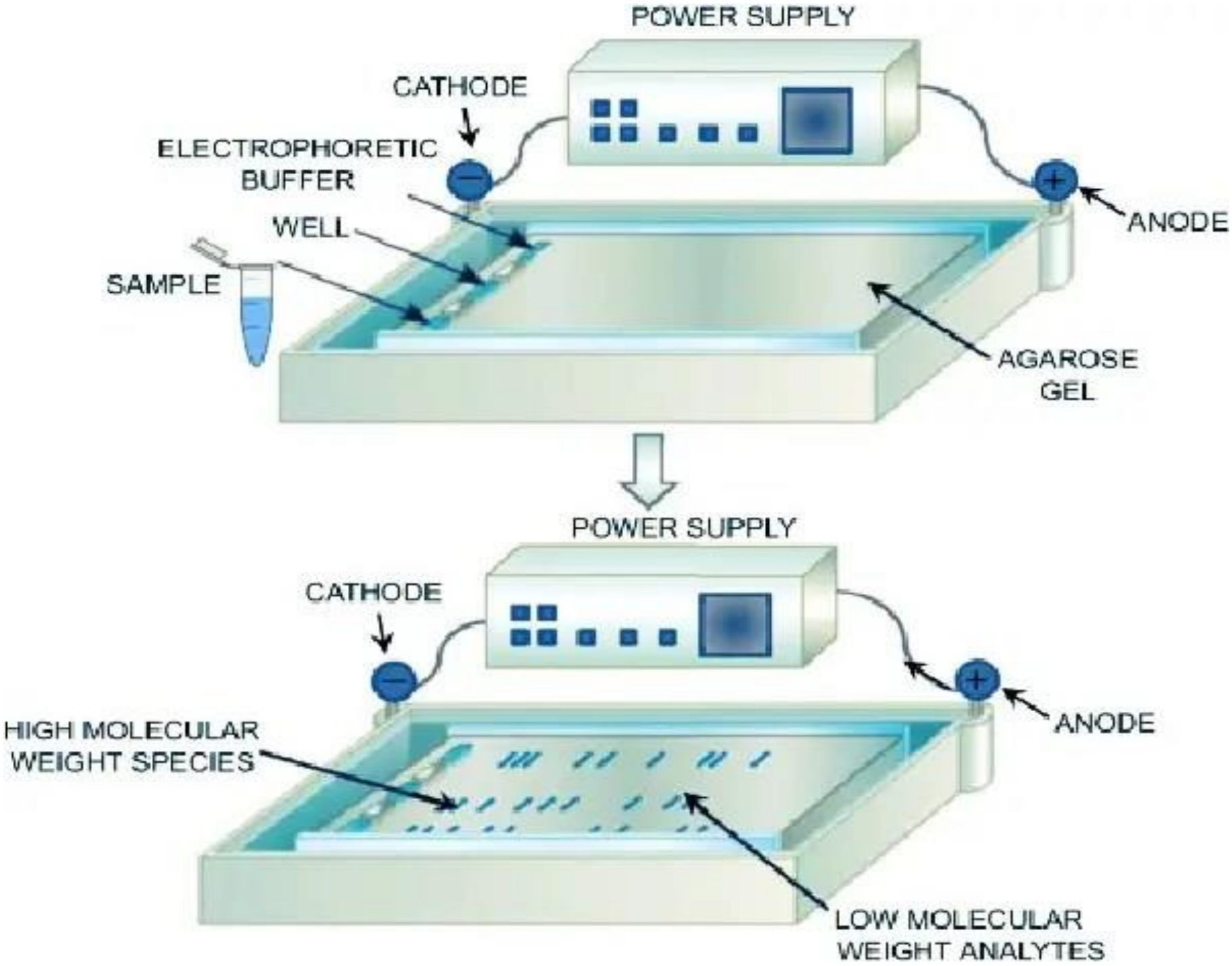
- **Detection and Quantification**
- Staining with a dye or autoradiography (for radioactive samples) helps in the detection of the separated components.
- Quantification is done using a densitometer or by direct measurement using an optical detection system.
- For example, protein is fixed by precipitating in gel with acetic acid. Methanol helps prevent the diffusion of proteins from the gel during the staining process.

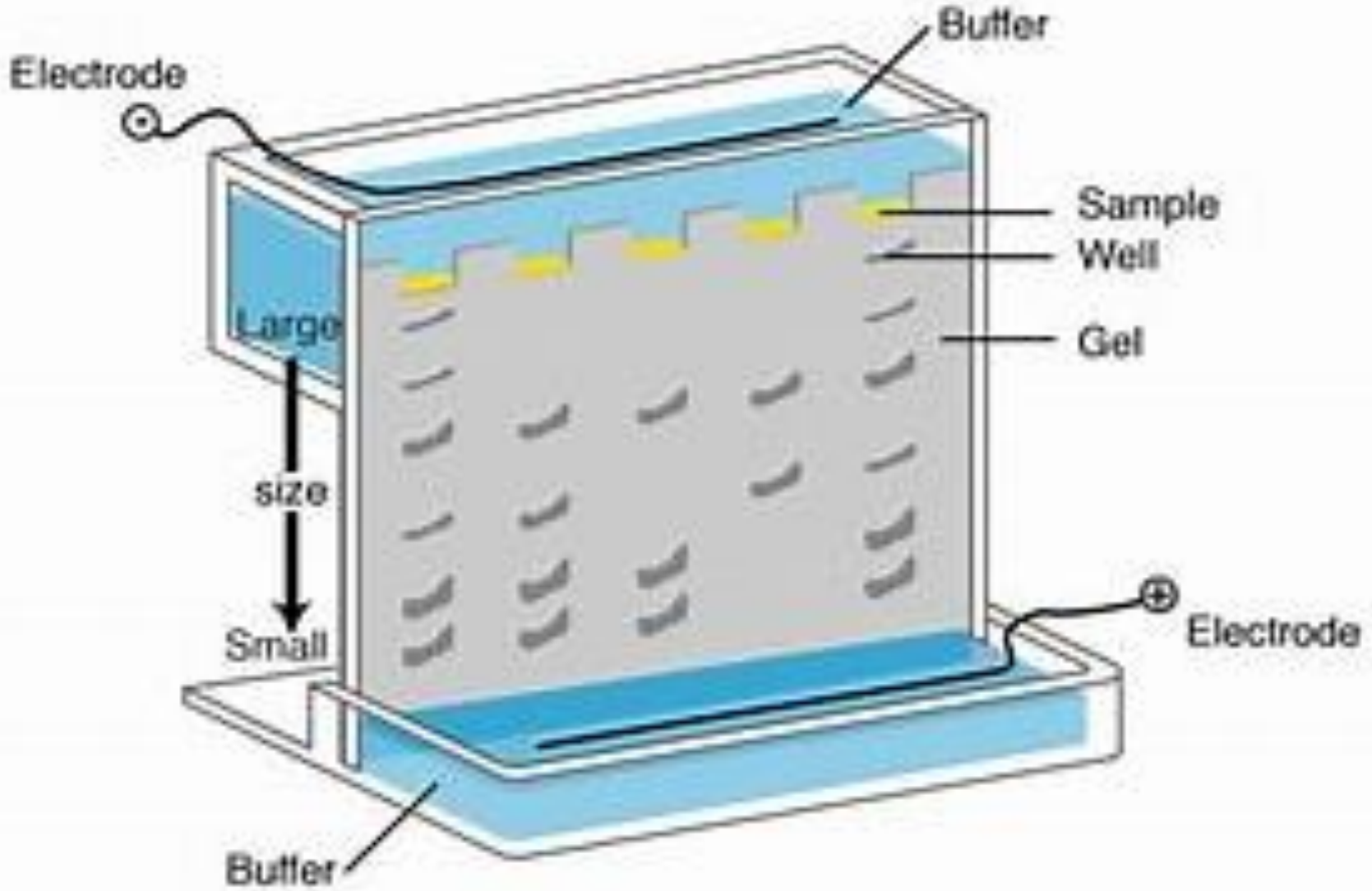
GEL ELECTROPHORESIS

- It is a technique used for the separation of Deoxyribonucleic acid(DNA), Ribonucleic acid (RNA) or protein molecules according to their size and electrical charge using an electric current applied to a gel matrix.
- Types of Gel:
  - Agarose gel.
  - Polyacrylamide gel.

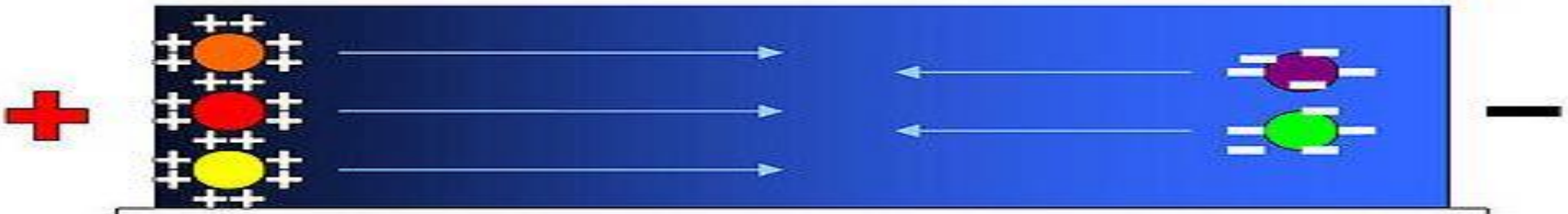
GEL ELECTROPHORESIS



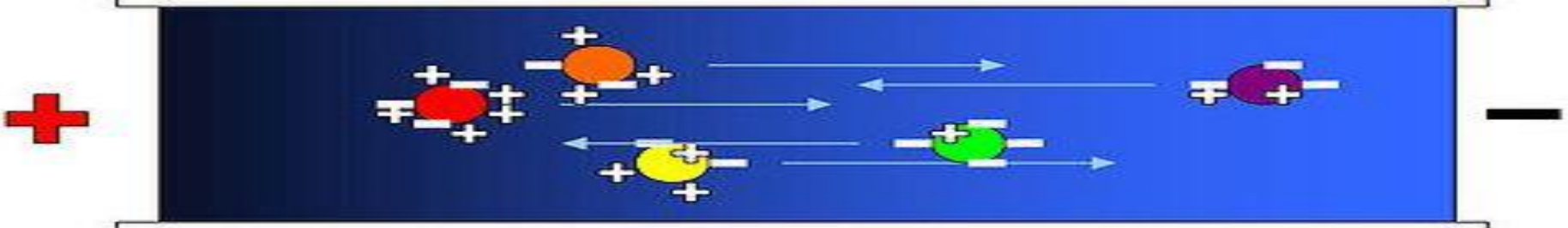

POWER SUPPLY
CATHODE
ELECTROPHORETIC BUFFER
WELL
SAMPLE
ANODE
AGAROSE GEL
POWER SUPPLY
CATHODE
ANODE
HIGH MOLECULAR WEIGHT SPECIES
LOW MOLECULAR WEIGHT ANALYTES


Buffer
Electrode
Sample
Well
Gel
Large
size
Small
Electrode
Buffer

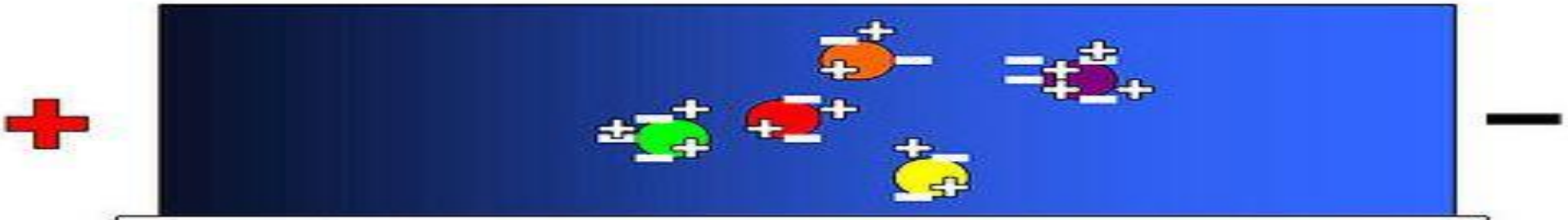
Stable pH gradient



At low pH, most proteins have a positive charge while at high pH, most proteins have a negative charge.



When an electric field is present, the cathode and anode ends pull the proteins to their isoelectric point where each individual protein possesses a neutral charge.



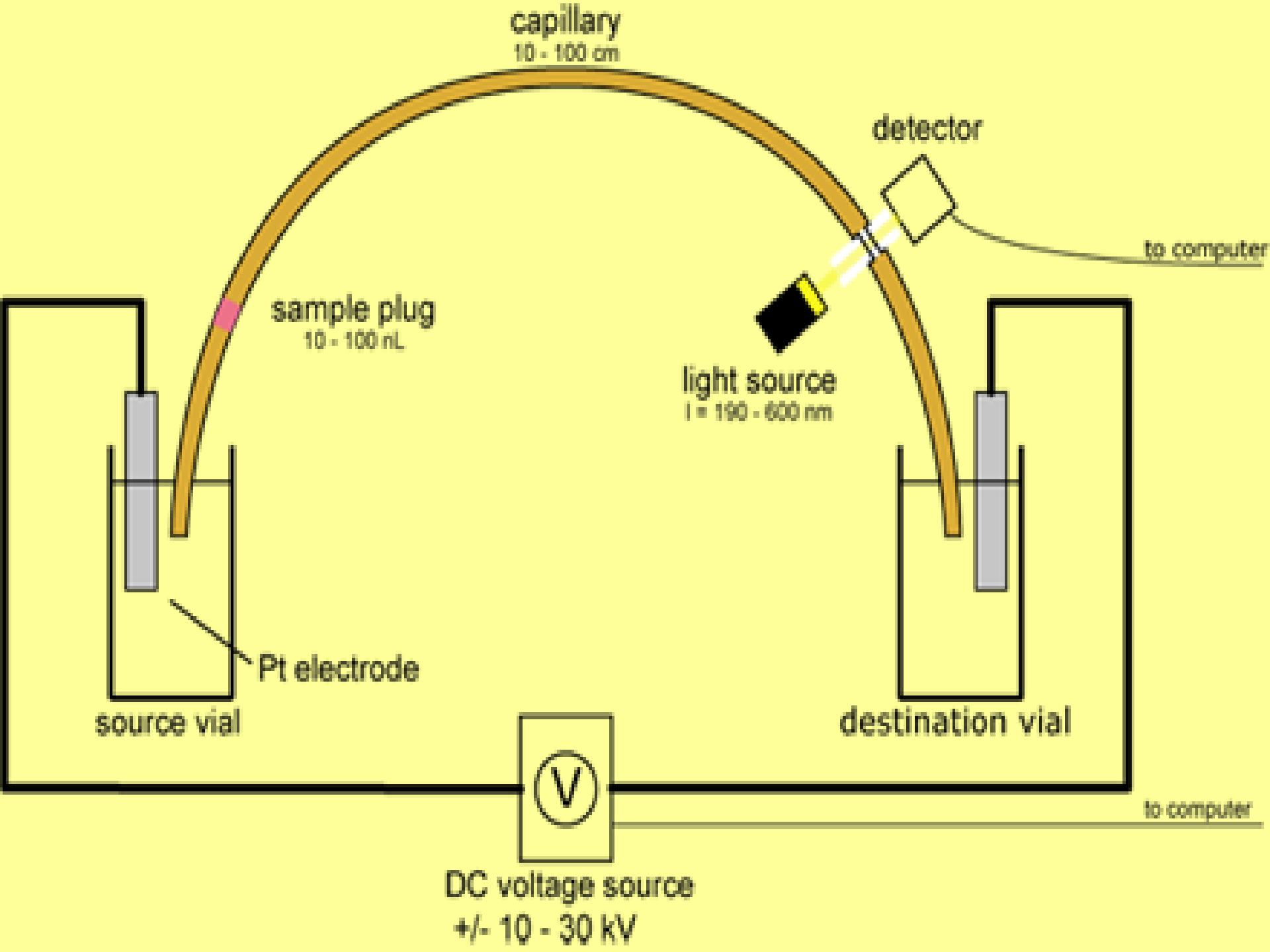
The proteins stopped migrating because they've reached their isoelectric point at a unique pH level.

Capillary Electrophoresis

. electrophoresis, is the technique of performing electrophoresis in buffer-filled, narrow-bore capillaries, normally from 25 to 100 pm in internal diameter .

- The capillary is filled with electrolyte solution which conducts current through the inside of the capillary. The ends of the capillary are dipped into reservoirs filled with the electrolyte.

- Electrodes (platinum) are inserted into the electrolyte reservoirs to complete the electrical circuit


capillary
10 - 100 cm
detector
to computer
sample plug
10 - 100 nL
light source
l = 190 - 600 nm
Pt electrode
source vial
destination vial
V
to computer
DC voltage source
+/- 10 - 30 kV

Uses of Electrophoresis

It is applied for routine laboratory experiments, disease diagnosis, research-oriented separations and identification.

Similarly, it is used in various other fields, like forensics, agriculture, pharmaceutical, foods, etc.

Application

- Determination of gene sequence
- Isolation of entire chromosome.
- separation and characterization of protein.
- DNA fingerprinting evidence.
- Restriction mapping of DNA

References

- Biophysical chemistry- Upadhyay & Nath
- Instrumental analysis- Skoog and Holler
- www.Slideshare.com

www.authorstream.com