Electrophoresis

Meaning of Electrophoresis: is a technique that separates compounds such as inorganic anions and cations, amino acids, drugs, vitamins, carbohydrates, peptides, proteins, nucleic acids, nucleotides, polynucleotide, lipoproteins, hemoglobin and numerous other species to individual components .

Electro = electrical field , phoresis = migration.

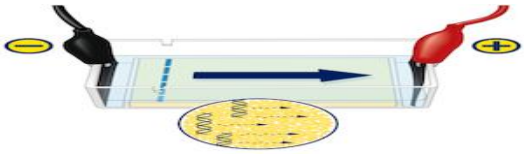
Definition of Electrophoresis: is migration of charged particles or molecules in a medium under the influence of an applied electric field.

Principle of Electrophoresis: a separation method based on the **differential rates** of migration of charged species in a liquid (gel) medium under the influence of electric field. Using two opposing electrodes. Electrophoresis uses the electrochemical cell the cathode is the negative electrode where reduction occurs and cations (positive ions) migrate to. Consequently, the anode is the positive electrode where oxidation occurs and anions (negative ions) migrate to.

- ☺ When electricity is applied, the molecules start moving to respective electrodes but the movement is influenced by molecular weight of the molecule so when a mixture is placed on the paper or gel, different bands are seen along the paper or gel after the process.
- ☺ those molecules with higher m.wt move slower while those molecules with small m.wt move faster

Factors that affecting on rate of migration:

i. the molecular weight ,Size and shape
ii. Net electrical charge of the molecular
iii. Electrical field strength
iv. Properties of the supporting medium
v. Migration time

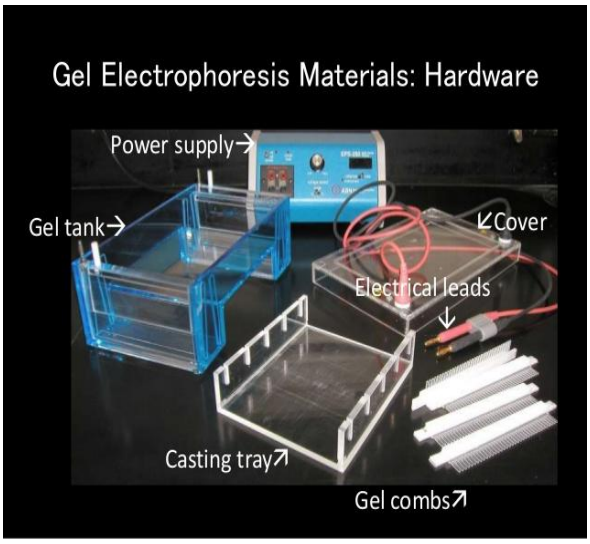


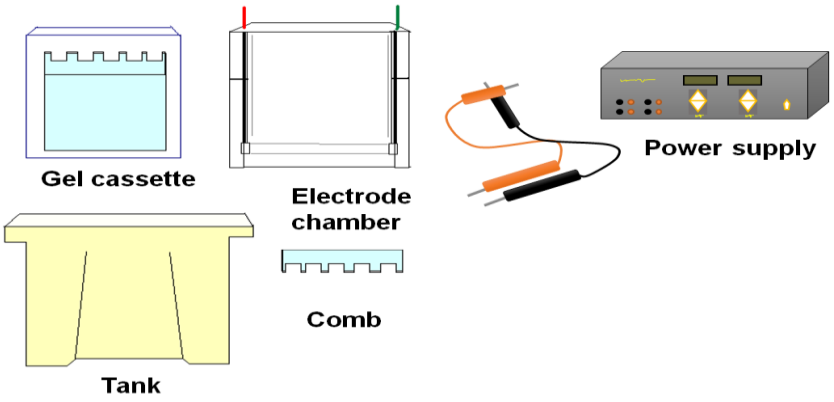
electrophoresis components

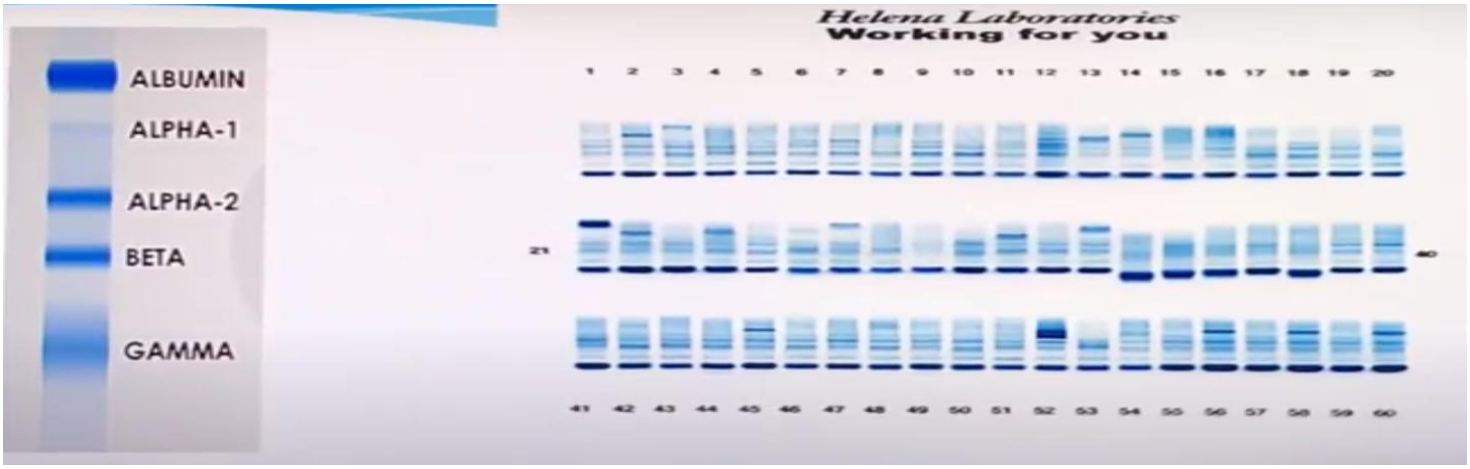
1- power supply (electric field.(DC) and Electrical leads **red + positive** connected with anode , black or blue **- negative** connected with cathode

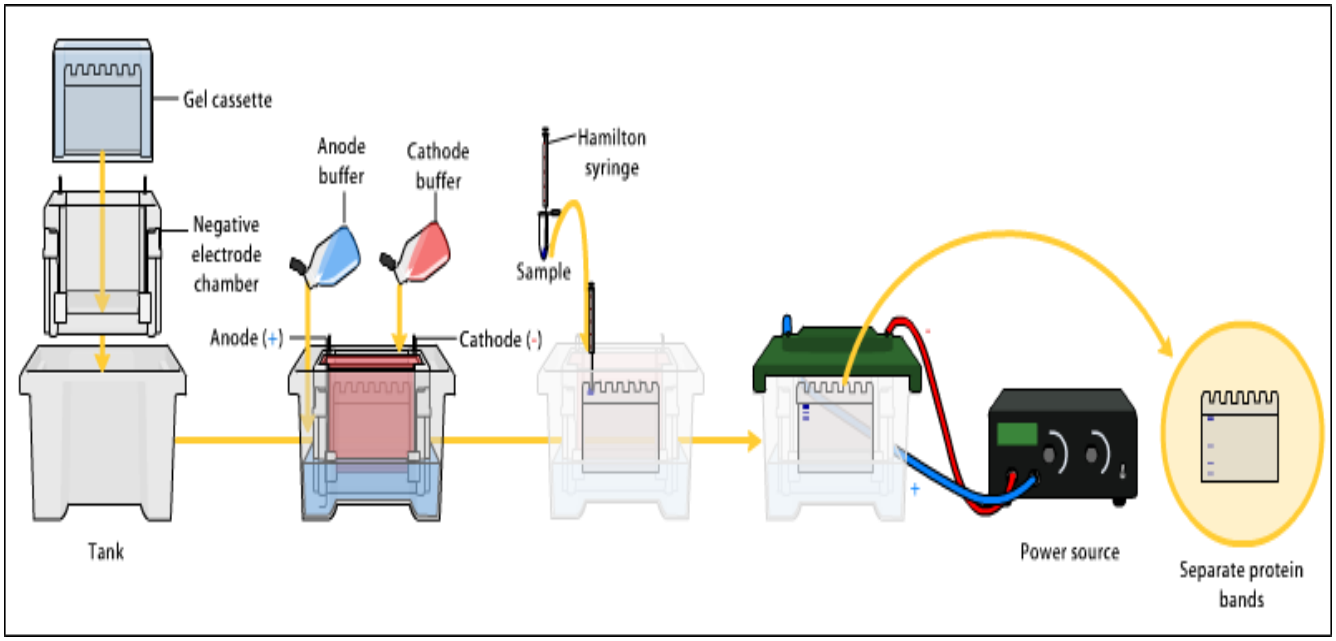
2- tank or gel box
   a- gel cassette \ tray b- electrode chamber c- gel comb d- sample e- buffer f- supporting medium g- cover

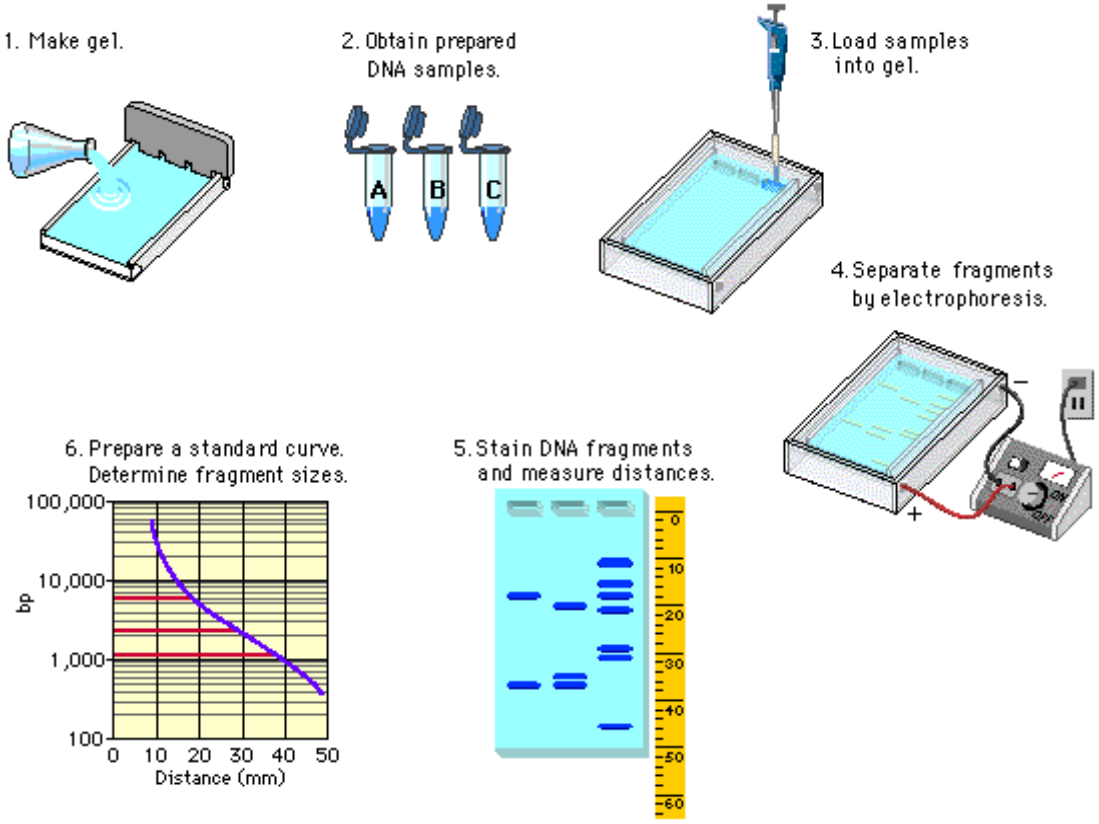
3- detecting system


Gel Electrophoresis Materials: Hardware
Power supply
Gel tank
Cover
Electrical leads
Casting tray
Gel combs


Gel cassette
Electrode chamber
Power supply
Comb
Tank


Helena Laboratories
Working for you
ALBUMIN
ALPHA-1
ALPHA-2
BETA
GAMMA


Gel cassette
Negative electrode chamber
Anode buffer
Cathode buffer
Hamilton syringe
Sample
Anode (+)
Cathode (-)
Tank
Power source
Separate protein bands


1. Make gel.
2. Obtain prepared DNA samples.
A
B
C
3. Load samples into gel.
4. Separate fragments by electrophoresis.
6. Prepare a standard curve. Determine fragment sizes.
100,000
10,000
1,000
100
bp
0 10 20 30 40 50
Distance (mm)
5. Stain DNA fragments and measure distances.
0
10
20
30
40
50
60

Detecting system

Densitometer is an instrument for determining the degree of darkening of developed Photographic or radiographic film or degree of optical density of a photographic, Based on the use of a photoelectric cell to measure the Light transmission through a given area of the film.

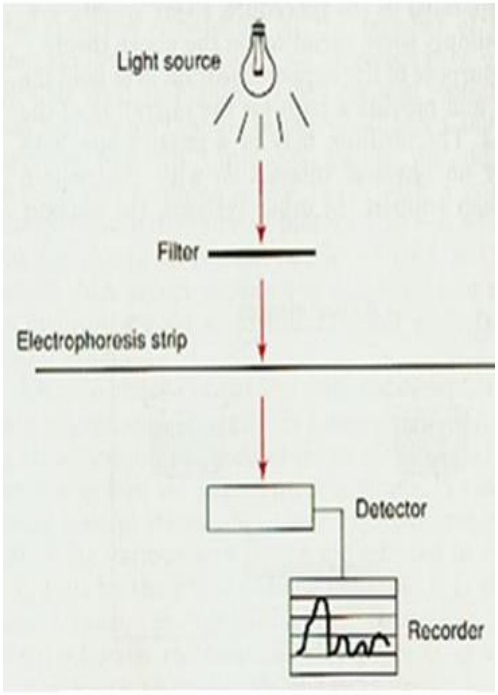
Densitometer

- ☺ Instrument for measuring the density of components (for example, protein fractions) separated by electrophoresis using light absorption or reflection.
- ☺ It determines the density of a sample placed between the light source and the photoelectric cell from differences in the readings
- ☺ The densitometer scans the stained electrophoresis strip and converts the intensity of the stain into graphical form

©©© as light beam passes through each stained band , the present transmission is recorded and a graph representation of the concentration .

Decrease T% ⟹ conc. Of sample is increase and seen as large peak on scan.

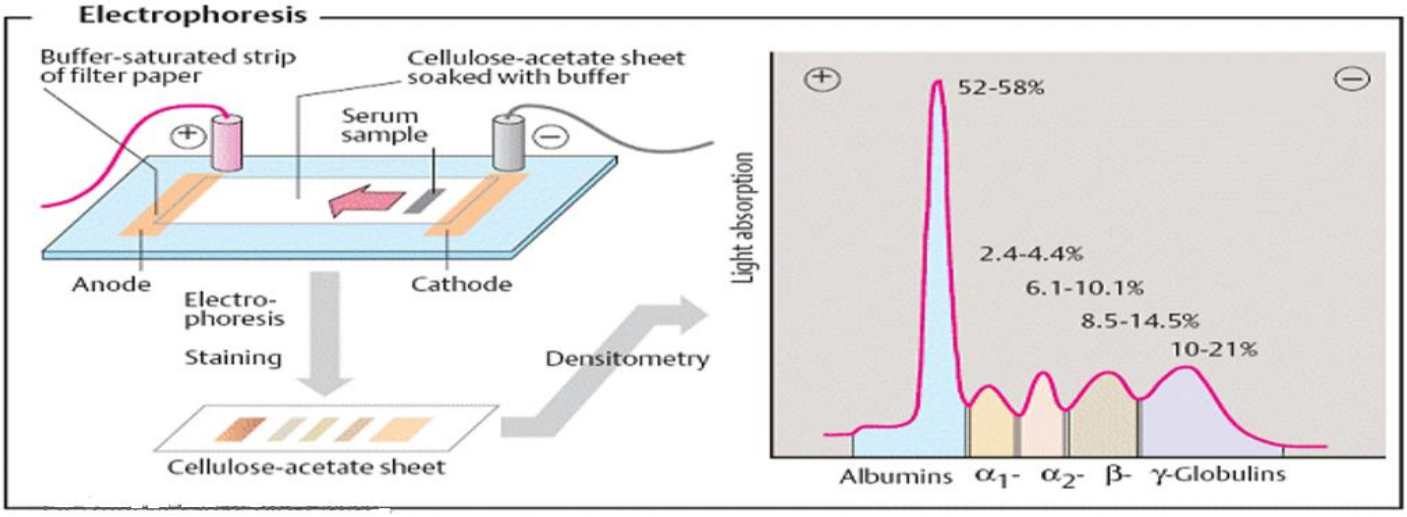
Increase T% ⟹ conc. Of sample is decrease and seen low peak or no peak



The densitometer scans the stained electrophoresis strip and converts the intensity of the stain into graphical form

Electrophoresis Densitometer

The densitometer ☺☺calculates the area under the curves (AUC) and expresses each as a percentage of the total



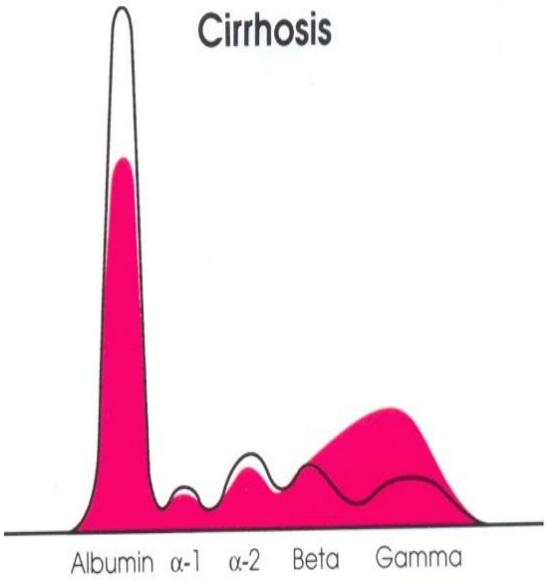
Graphical representation of the densitometer's scan of the electrophoresis strip.

It is usually performed by applying a small amount of serum to a strip of cellulose acetate or a garose and passing a current across it for a standard time. In this way, five main groups of proteins – namely albumin and the α1, α2, β and g globulins – may be distinguished after protein staining and may be visually compared with those in a normal control serum.

By electrophoresis, four major types of proteins.

- These are 1) albumin, 2) alpha globulins, 3) beta globulins and 4) gamma globulins.
- This test is useful for evaluation of patients who have abnormal liver function tests

If the gamma globulin fraction is elevated,



Nephritic Syndrome

This is an evaluation of the types of proteins high alph globulin that are present with in a patient's renal disease.