Al-Mustaqbal University
College of Healthcare and Medical Techniques
Intelligent Medical System Department

كلية التقنيات الطبية والصحية

قسم الانظمة الطبية الذكية

Lecture: (2)

Subject: Bio chemistry
Class: Second
Lecturer: Mcs.wafaa ghalib

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Biochemistry

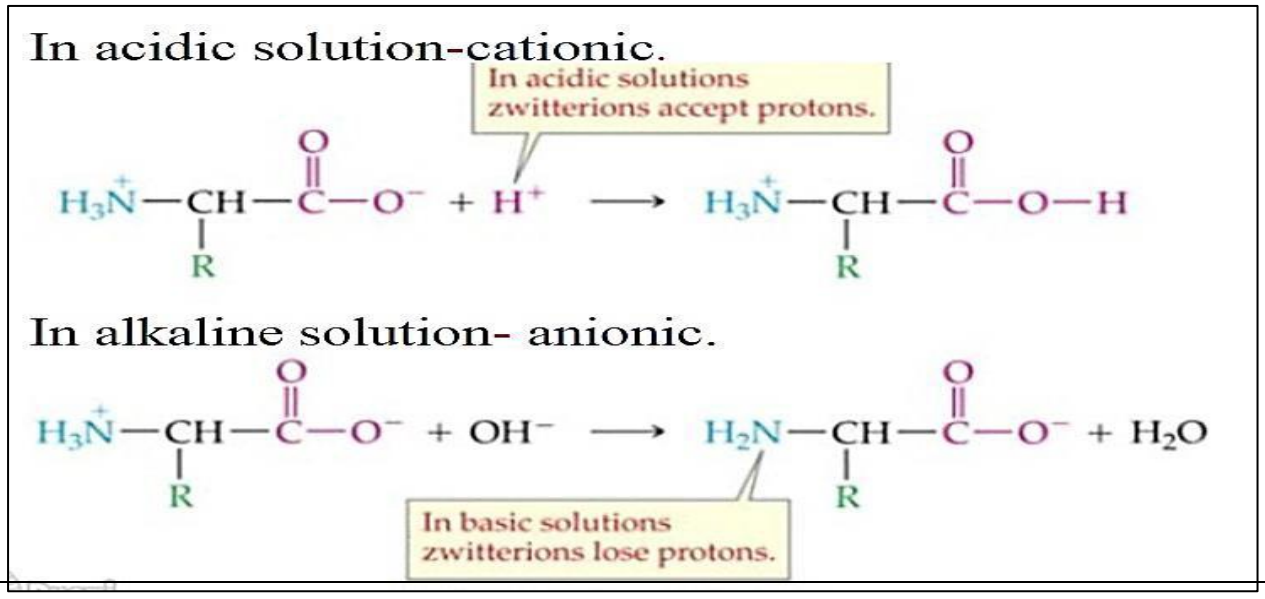
Electrophoresis

Electrophoresis is a separation technique that depends on the movement (migration) of charged particles such as proteins and nucleic acids in serum under the influence of an electrical current.

The principle of Electrophoresis

Each protein behaves as anionic protein (net negative charged) in alkaline medium and as cationic protein in acidic medium (net positive charged). Under the influence of electric field these charged proteins will migrate either to cathode or anode depending on the nature of their net charge.

- In serum protein electrophoresis, protein fractions are charged with negative charge by placed in alkaline solution (buffer).



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Factors effecting on a velocity of protein migration

1. The net electric Charge
2. Electric field strength
3. PH of buffer Solution
4. Molecular weight of the protein (size)
5. Nature of supporting medium

Types of supporting media in Electrophoresis

1. Starch Gel
2. Polyacrylamide Gel
3. Agar Gel
4. Cellulose Acetate Paper

- Serum proteins separates by electrophoresis into five distinct fractions: (albumin, α-1, α-2, β & γ globulins)

- Albumin are the fastest in the movement towards the anode, followed by α-1, α-2, βand finally γ, which is hardly moving because, decreasing its negative density.

Components of gel Electrophoresis

1. Plastic tank (Chamber)
2. Casting tray
3. Applicator (comb)
4. Cover
5. Power supply
6. Electrical leads (cathode and anode)

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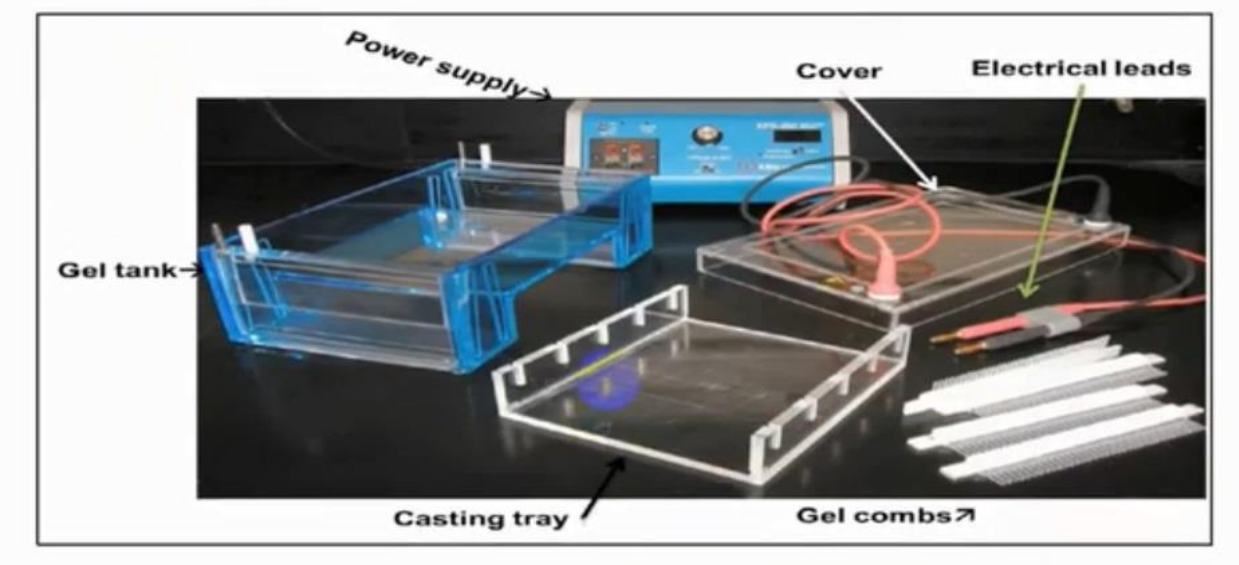


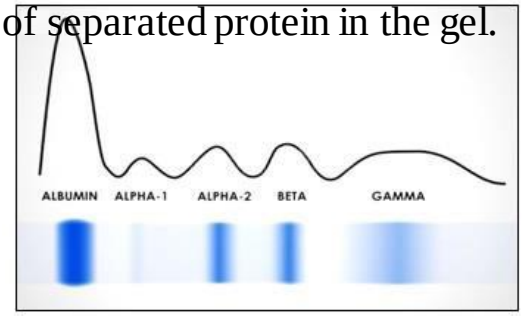
Solutions of protein electrophoresis

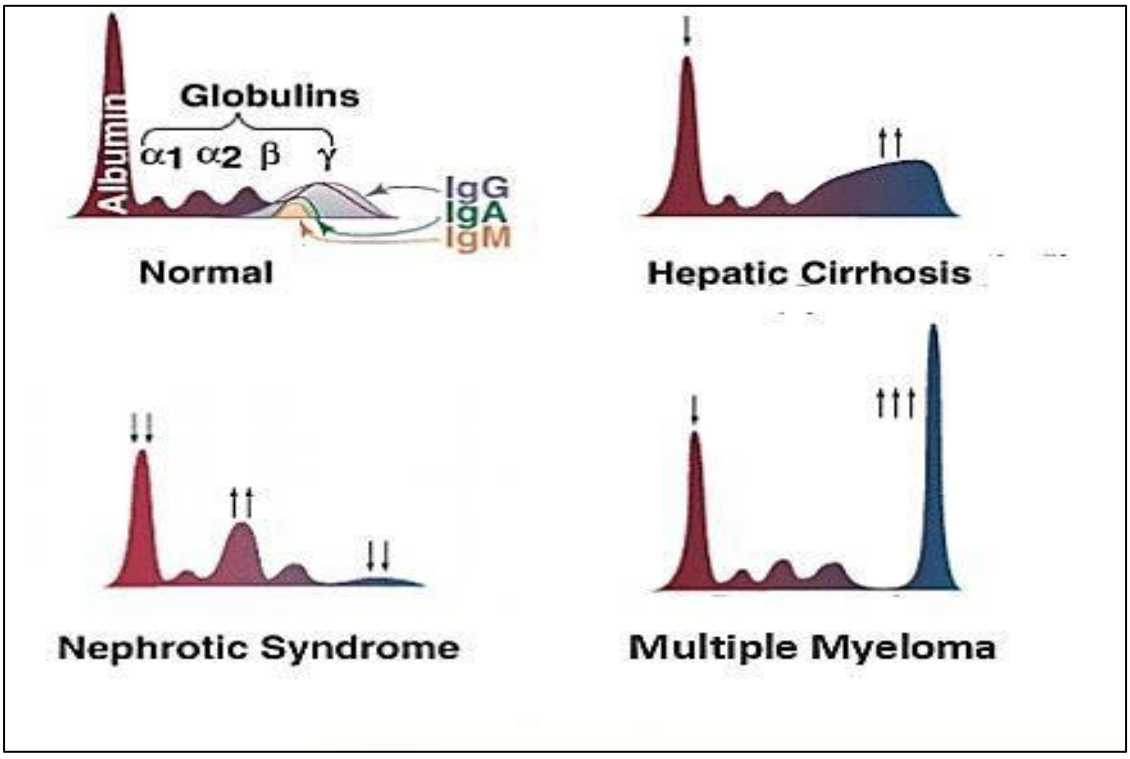
1. Buffer solution pH 8.6 (sodium barbitone and barbituric acid)
2. Fixed solution (0.05N NaOH)
3. Staining solution (Coomasie Brilliant Blue or amido black)
4. Destaining solution (20% methanol + 5% glacial acetic acid)

Procedure of gel electrophoresis

1. Prepare gel solution (agarose)
2. Insert comb in casting tray, pour gel solution into the tray and let it to cool for 20min then remove the comb from gel.
3. Gel (which contains a series of wells at the cathode end) placed inside the chamber and covered with a buffer solution.
4. Apply 10 µl of sample into gel wells by micropipette.
5. Adjustment power supply (100 volts, direct current) and separating time 30min.
6. After completion of run, switched off the power and remove gel from the casting tray using a spatula.
7. Dip the gel in fixed, staining and destaining solutions (10 min for each steps).
8. The evaluation can be qualitative (standards) and quantitative (densitometry)

- Densitometer is used for scanning of separated protein in the gel.





Cases	Albumin	α-1	α-2	β	γ
Nephrotic Syndrome	-	/	+	/	-
Liver Cirrhosis	-	-	/	+	+
Multiple Myeloma	-	/	/	/	++

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Normal values of the separate parts of the protein in the serum

1. Total protein = 6.0 – 8.0 gm/dl
2. Albumin = 3.5 – 5.0 gm/dl
3. Alpha – 1 = 0.17 – 0.33 gm/dl
4. Alpha – 2 = 0.42 – 0.90 gm/dl
5. Beta = 0.52 – 1.05 gm/dl
6. Gama = 0.71 – 1.65 gm/dl