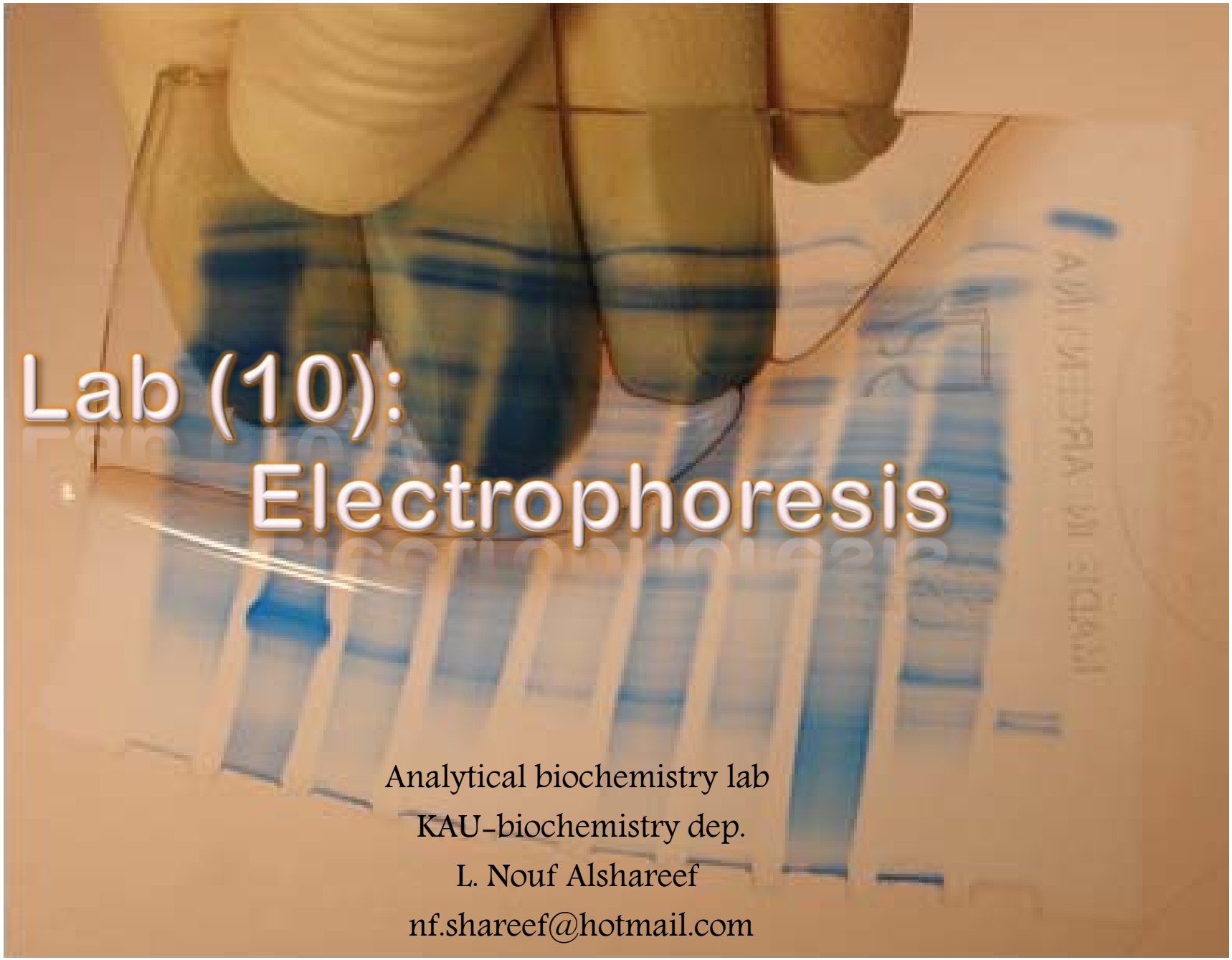


Lab (10): Electrophoresis

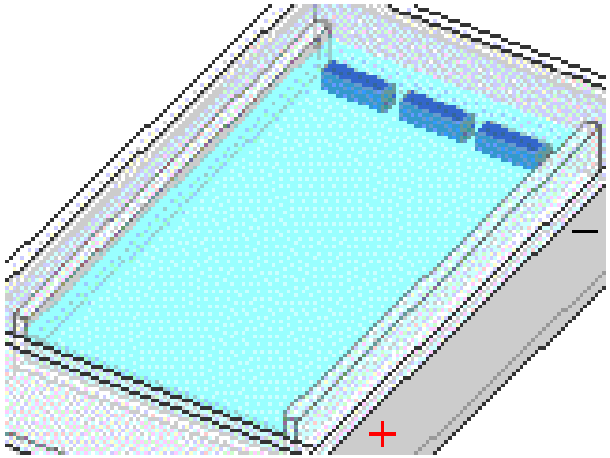
Analytical biochemistry lab
KAU-biochemistry dep.
L. Nouf Alshareef
nf.shareef@hotmail.com

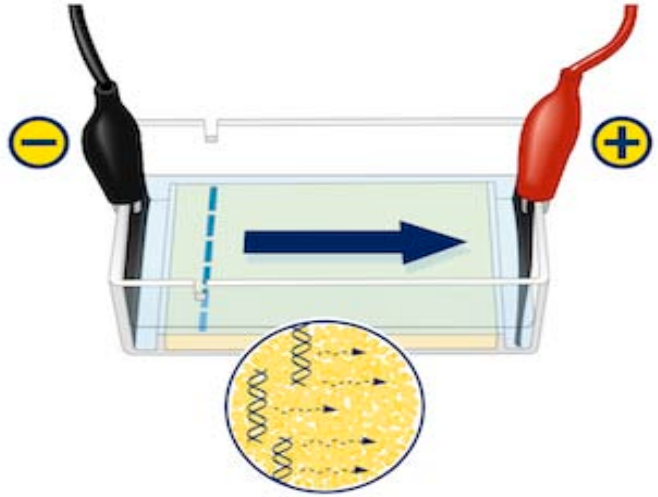
Gel electrophoresis

- is a technique used to separate charged molecule (DNA, RNA and protein) under the influence of an electrical field.

- **Electrophoresis** term refers to: the movement of particles through a porous matrix (gel) by electromotive force (EMF) according to their size (mass) and charge.

- Used as **analytical technique** and **preparative technique**

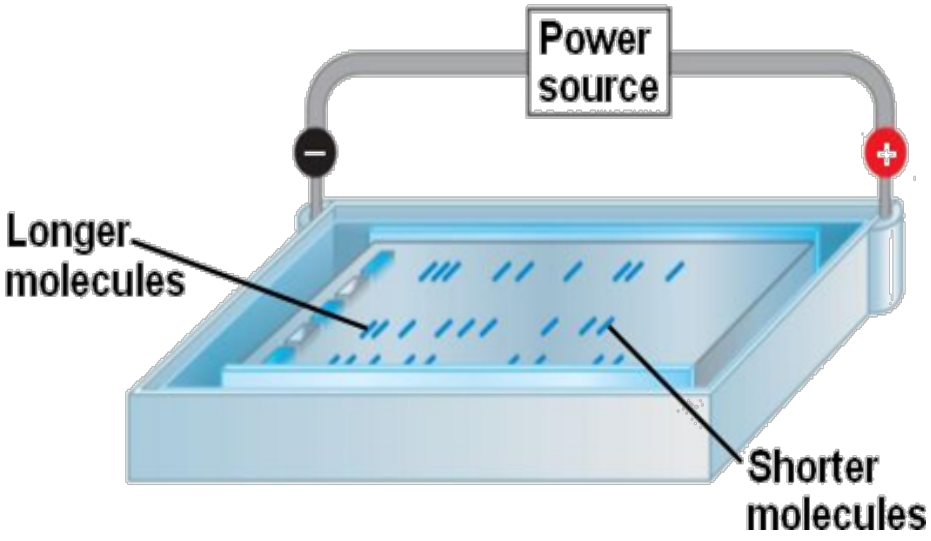




- Molecules move at different **rates** according to their weight (mass) and charge:

1- Negatively charged molecules **migrates** toward cathode (+) electrode
Positively charged molecule **migrates** toward **anode (-) electrode**

2- Small size (low M.wt) molecules **migrates faster**
Large size (high M.wt) molecules **migrates slower**



The rate of migration is also depends on:

- Strength of electrical field
- **Sample:** charge, size, shape and ionic strength
- **Medium (buffer)**: pH, viscosity, temperature and ionic strength
- **Supporting material**: Gel concentration

Example:

High voltage electrical field cause rapid movement but poor separation

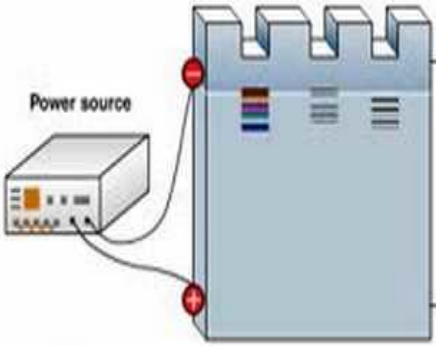
Electrophoresis can be:

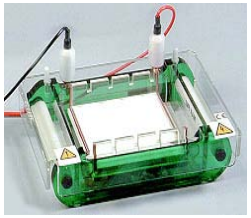
Horizontal or slab gel

Gel is poured in plat

Vertical

Gel poured between two glasses





Modular Tank with PAGE Insert

Blotting Module

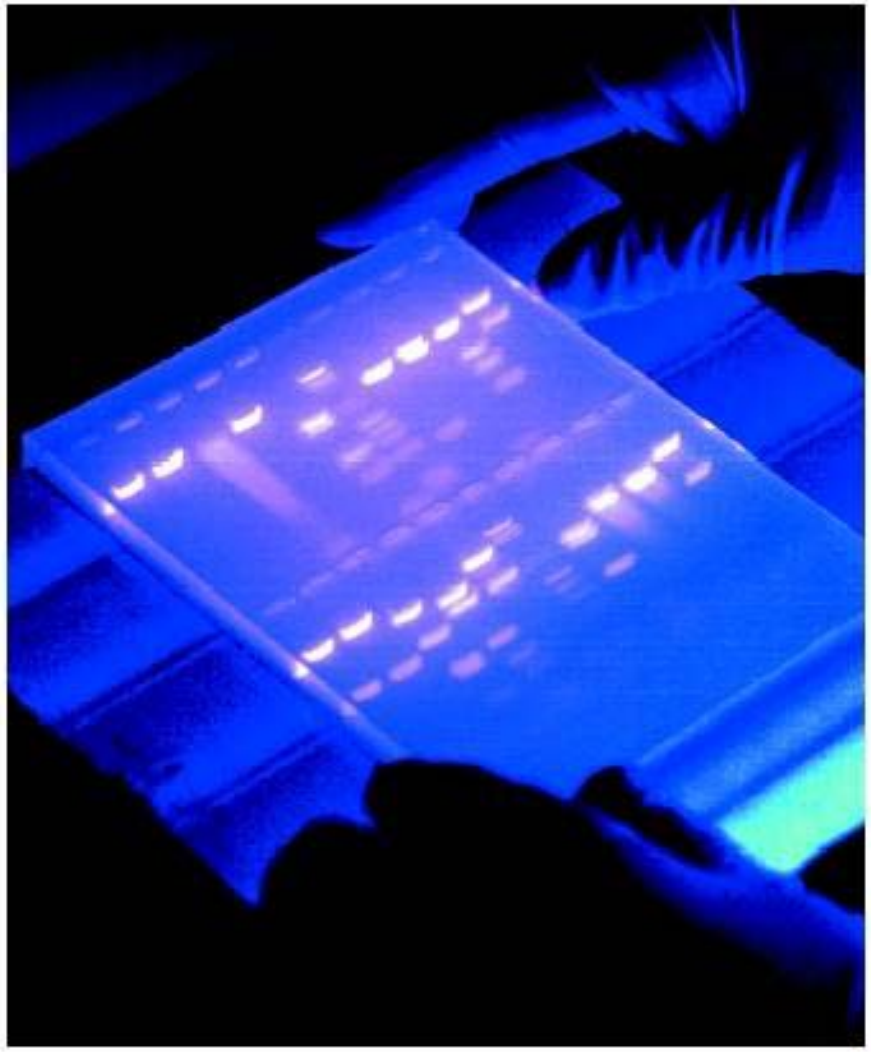
2D Module

Casting Stand and Combs

EQUIPMENTS AND MATERIALS

Material (chemicals)

- Supporting material (gel)
- Medium (buffer)
- Dyes
- Sample
- Marker

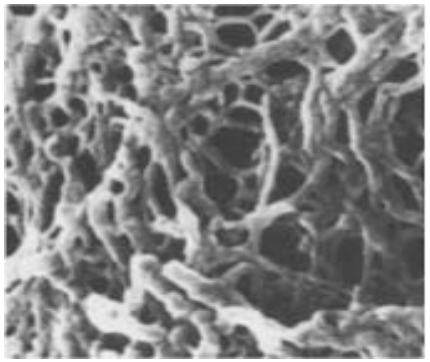


1- Supporting medium:

- Paper (filter paper)
- Cellulose acetate
- Starch gel
- Agarose gel
- Polyacrylamide gel electrophoresis (PAGE)

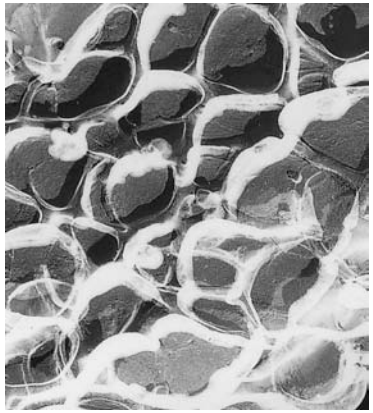
- **Agarose and PAGE are commonly used.**

Agarose gel:



- porous material that sample move though it.
- Used in DNA and protein
- Low range of conc. can prepared (0.5-3%)

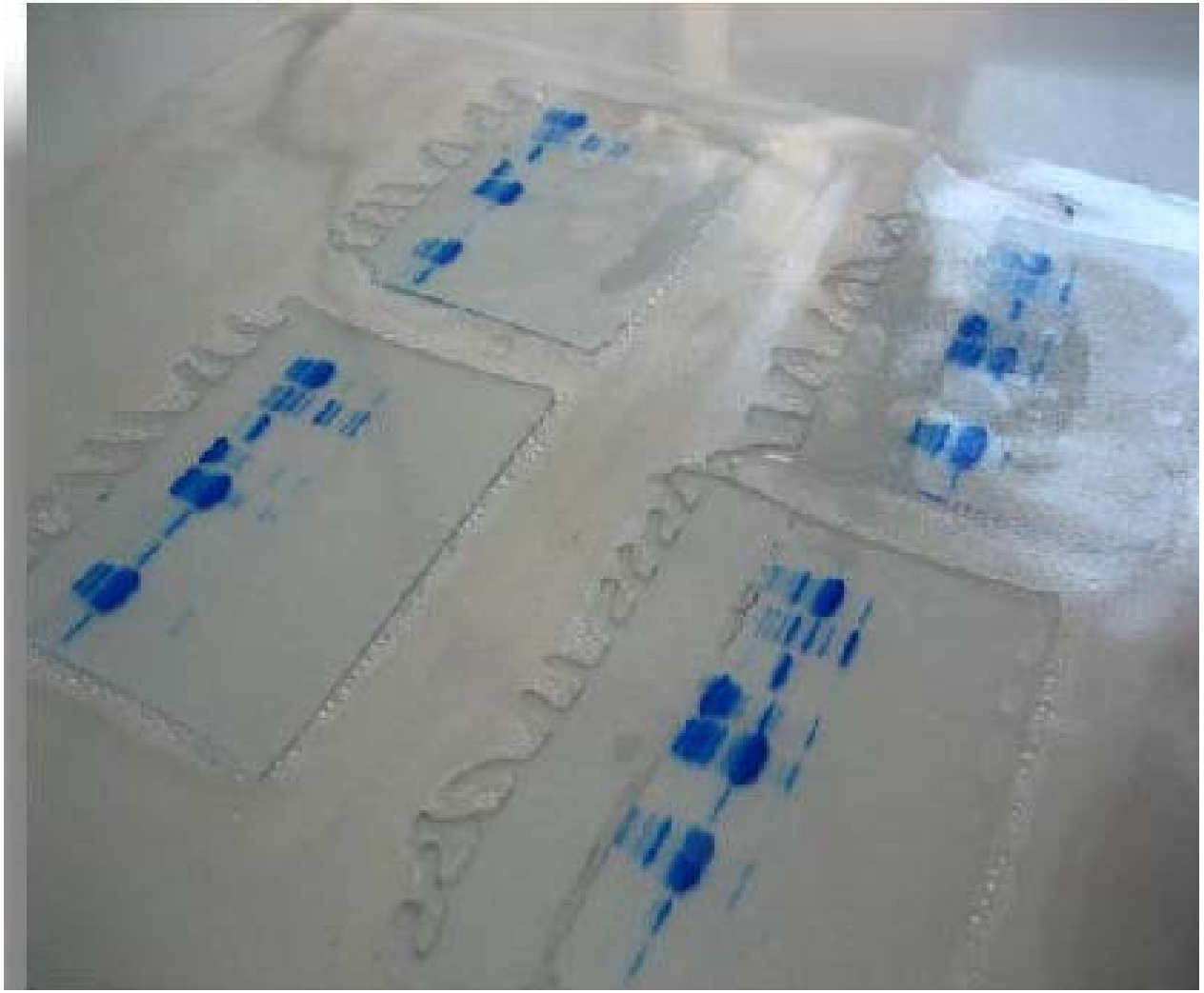
Polyacrylamide gel electrophoresis (PAGE):



- Is also porous consists of two material: acrylamide + bisacrylamide

  (cross link)
- Used in: DNA sequencing, protein, assessing M.wt of protein.
- High range of conc. can prepared: (2-20%) giving small pore size.

Polyacrylamide gel



2- Buffer
(ionic strength, pH)

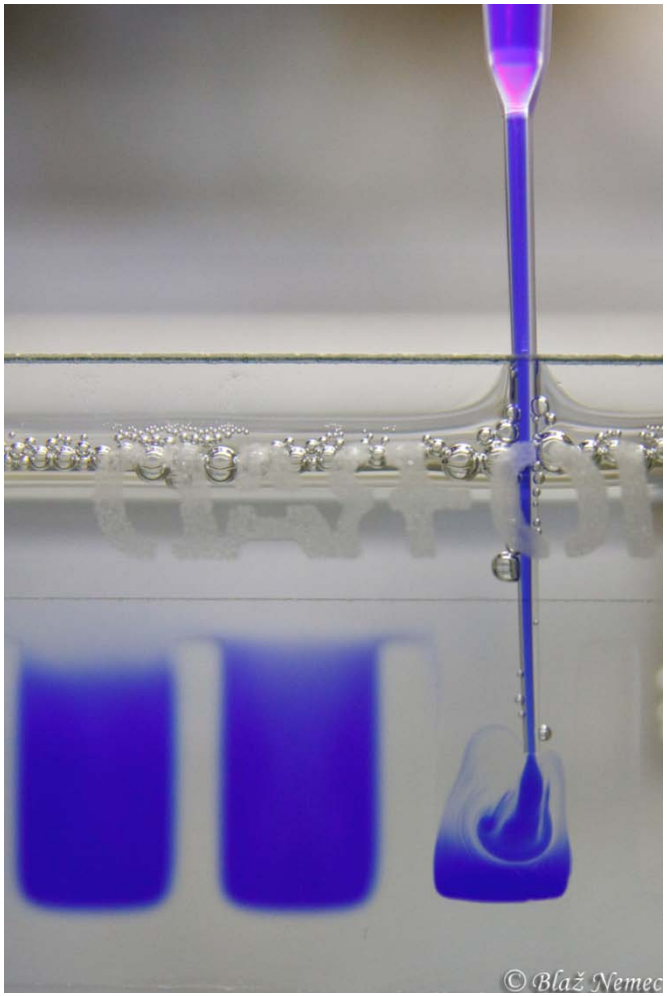
- Function: carry electric current.
- Ionic strength = concentration of ions in solution
- When ionic strength of the buffer increased this lead to form sharp zones, but decrease the migration rate.

3- Dyes Visualization

Two types of dye are used:

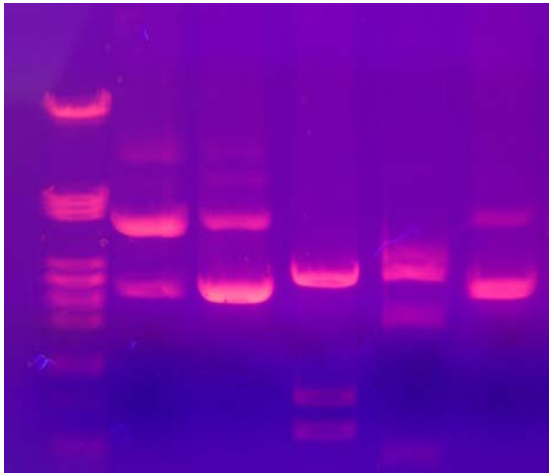
1- Tracking dye or loading dye:

- Used to monitor the migration, help in sample loading
- Bromophenol blue

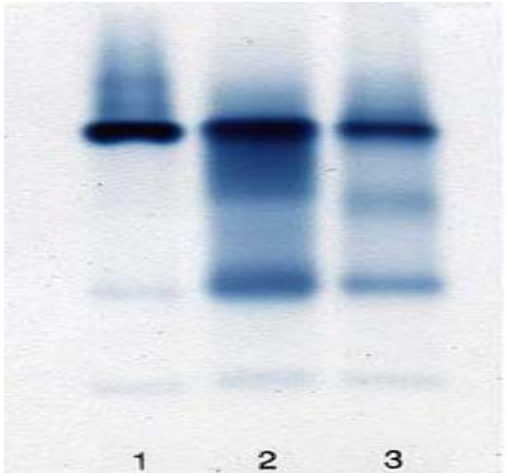


2- Visualization dye:

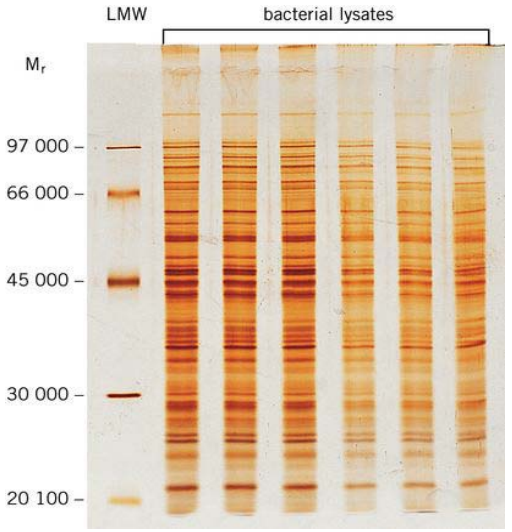
- Ethidium bromide (DNA, RNA)
- Silver
- Coomassie blue dye (protein).



Ethidium bromide



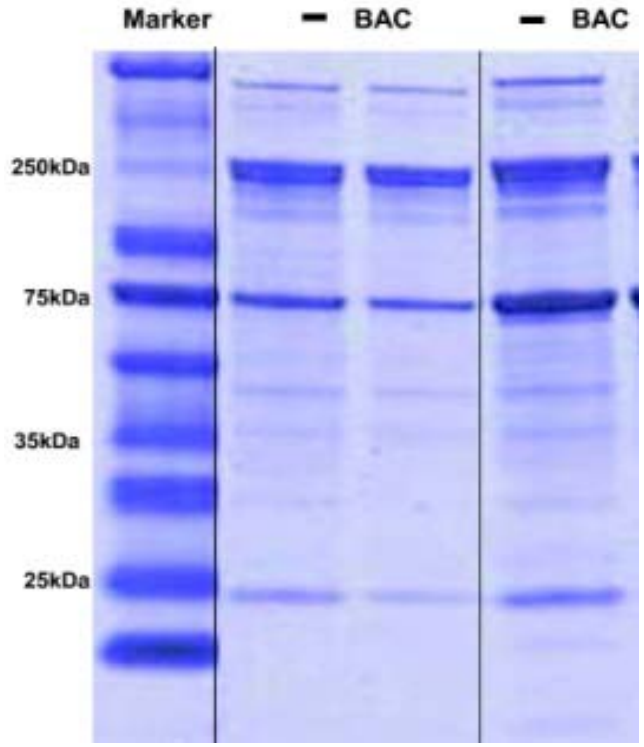
coonassie blue dye

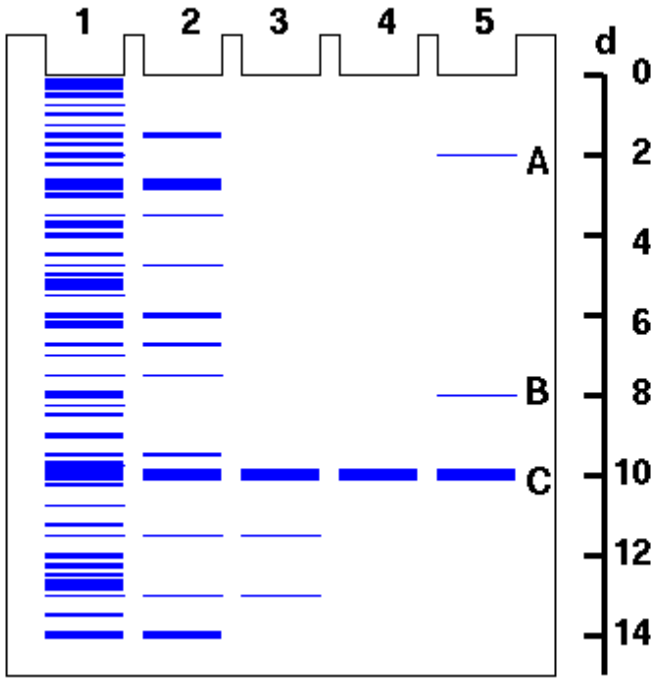


Silver staining

4- Molecular weight size marker

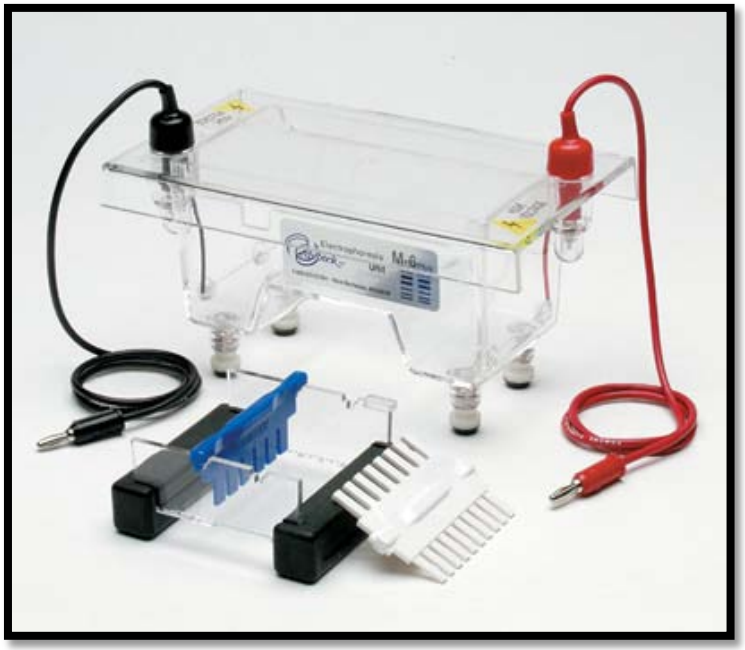
- mixture of molecules of known sizes may be protein (Da) or DNA (bp).

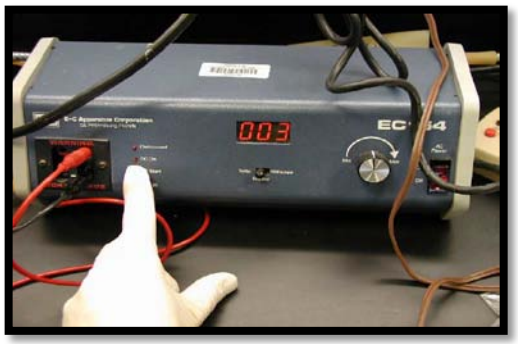




Equipments

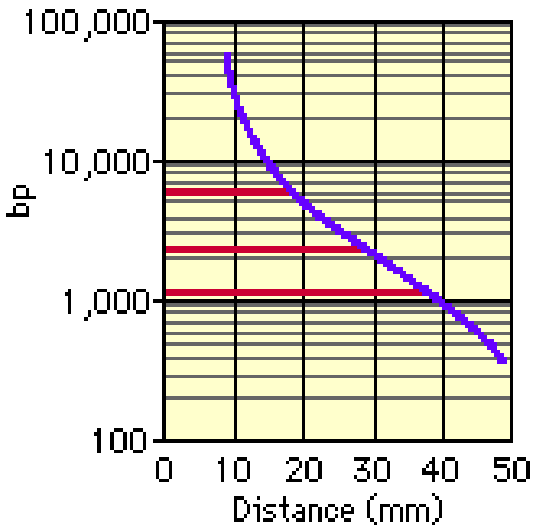
- Casting tray
- Tank with cover
- Comb
- Power supply

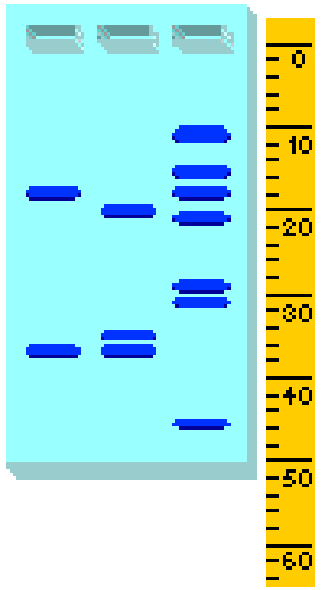




Determination of Molecular Weight

- using PAGE of proteins or agarose gel for DNA
- known M.wt (marker) is used along with sample
- Run electrophoresis.
- plot a standard curve of distance migrated vs. log Mwt





Lab practice:
Immunoelectrophoresis

- Immunoelectrophoresis (IEP), gamma globulin electrophoresis, immunoglobulin electrophoresis or Serum protein electrophoresis (SPEP)

- Is screening test measures the major blood proteins.
- Used to evaluate, diagnose, and monitor a variety of diseases.
- Levels of blood proteins increase or decrease due to disease.

- **Serum proteins are separated into five fractions**:
  albumin, α_1, α_2, β, and gamma proteins.

Serum Proteins

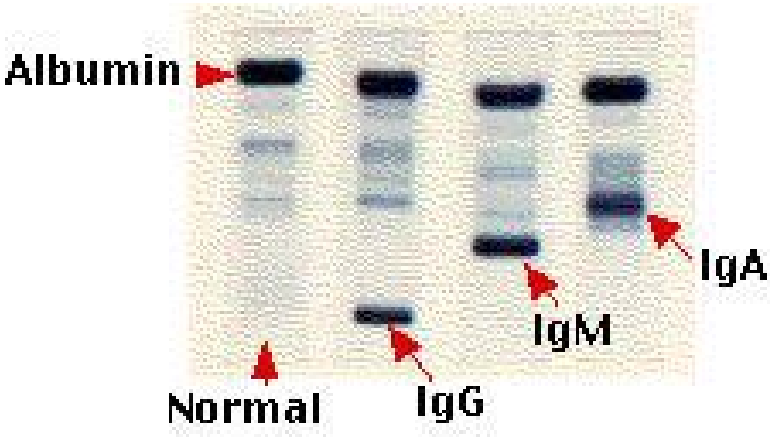
- Proteins make up 6-8% of blood.

  50% **serum albumin**, 50% variety of **serum globulins**.

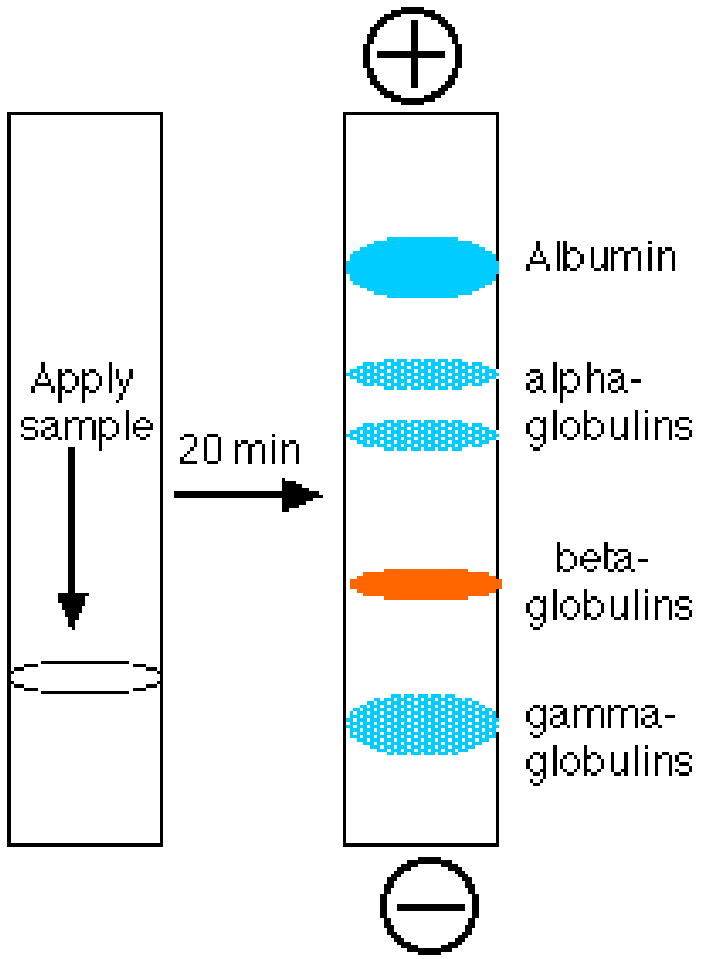
- **How to prepare serum:**

  Blood withdraw >>>allows to clot>> > clear fluid called serum is separated out.

- **So, serum** has same components of blood plasma without fibrinogen and other clotting factors.



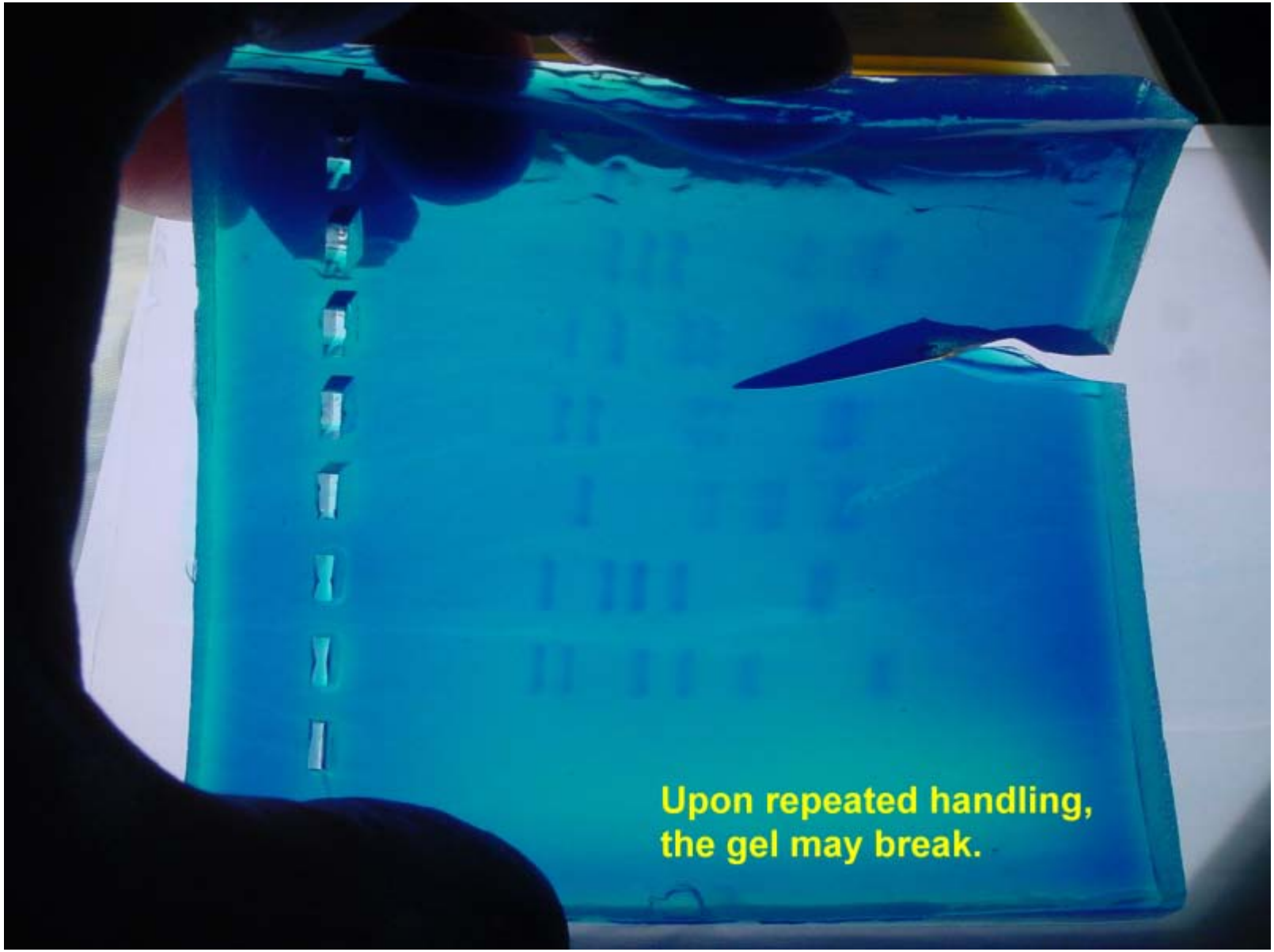
- At pH 8.6 all proteins are negatively charged, but some more strongly than others.
- serum proteins move toward the positive electrode.
- The separated proteins appear as distinct bands.
- They migrate in the order
  - **Albumin**
  - **alpha**
  - **beta globulins**
  - **gamma globulins**.



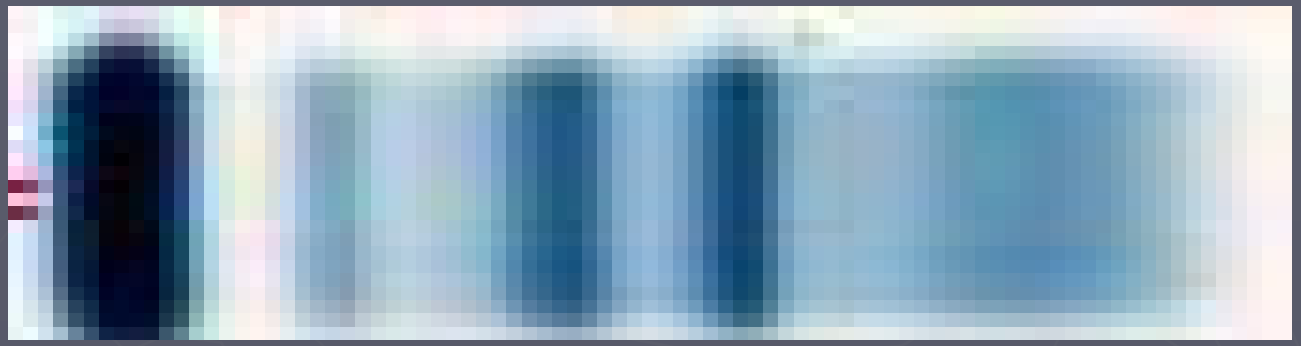
Separating serum proteins by electrophoresis

Procedure:

1. Prepare 1% agarose gel (1g agarose +100ml buffer)
2. Prepare sample by mixing 1µl loading dye (BPB) + 5µl serum
3. Load samples in gel wells (well should be in -ve electrode side)
4. Switch on power supply at 90volt and run for 30min.
5. Stain gel by soaking in commasie blue for 5min
6. De-stain gel by soaking in de-staining solution for 10-15min
7. Identify the bands resluted.


Upon repeated handling,
the gel may break.

Serum Protein Electrophoresis



+ α_1 α_2 β_1 & β_2 γ -

Albumin

α_1AT

Haptoglobin
α_2 macroglobulin
HDL

LDL
Transferrin
C3

Immunoglobulins &…
Fibrinogen, CRP, LDH.

Liver Proteins

Lymphoid Proteins

Result

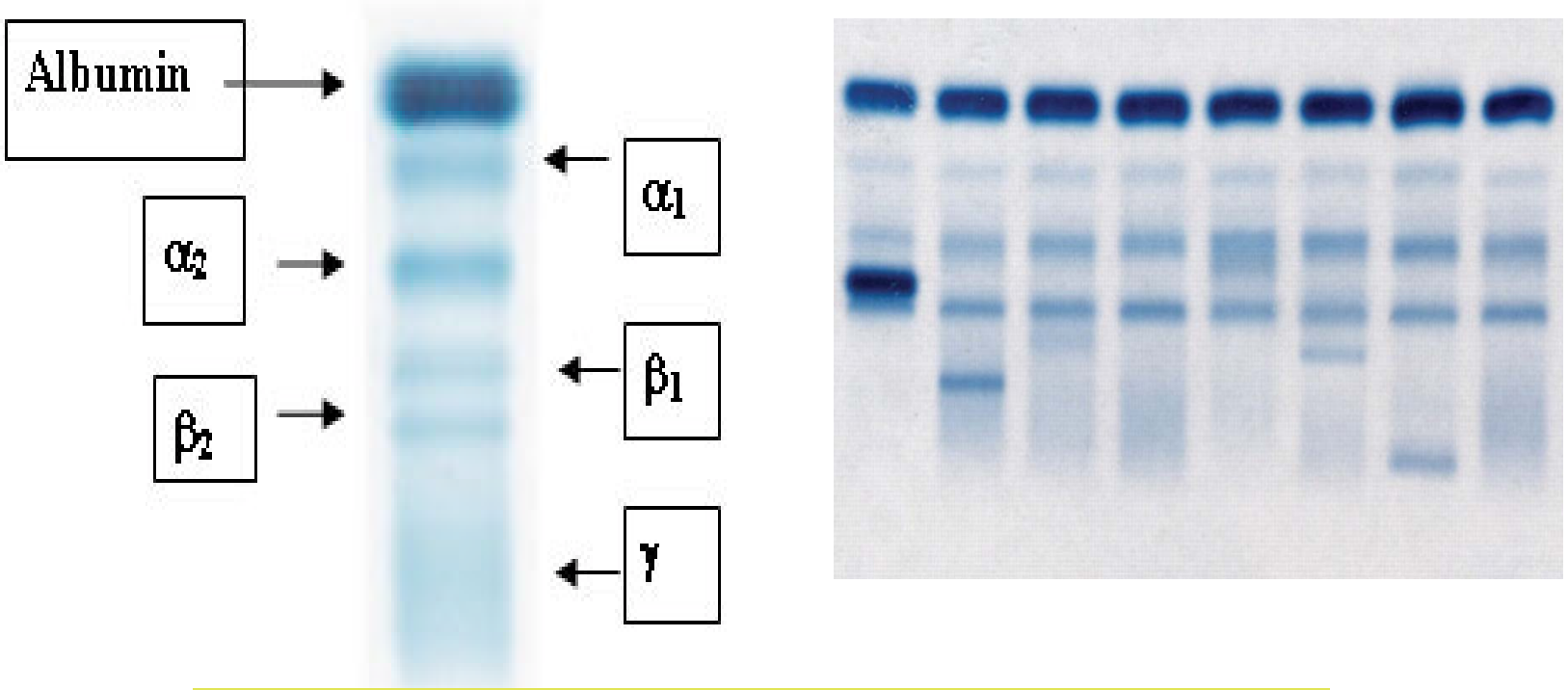
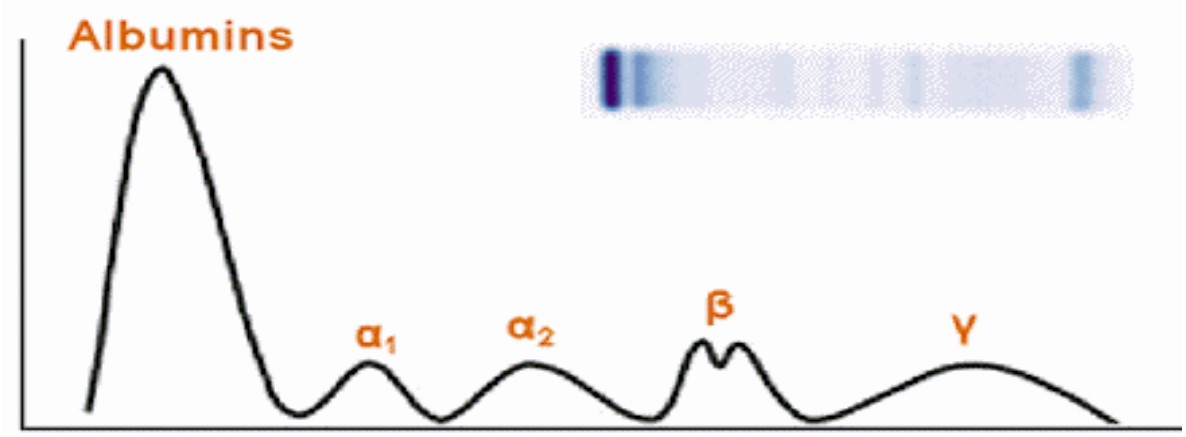
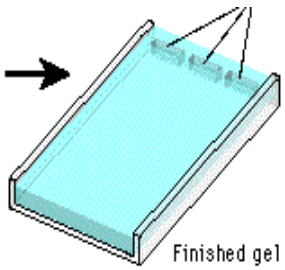


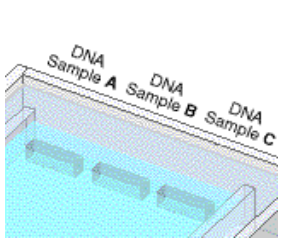
Figure 1: **Normal electrophoretic graph and Blood Proteins**

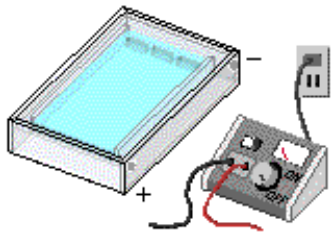


Important Terms

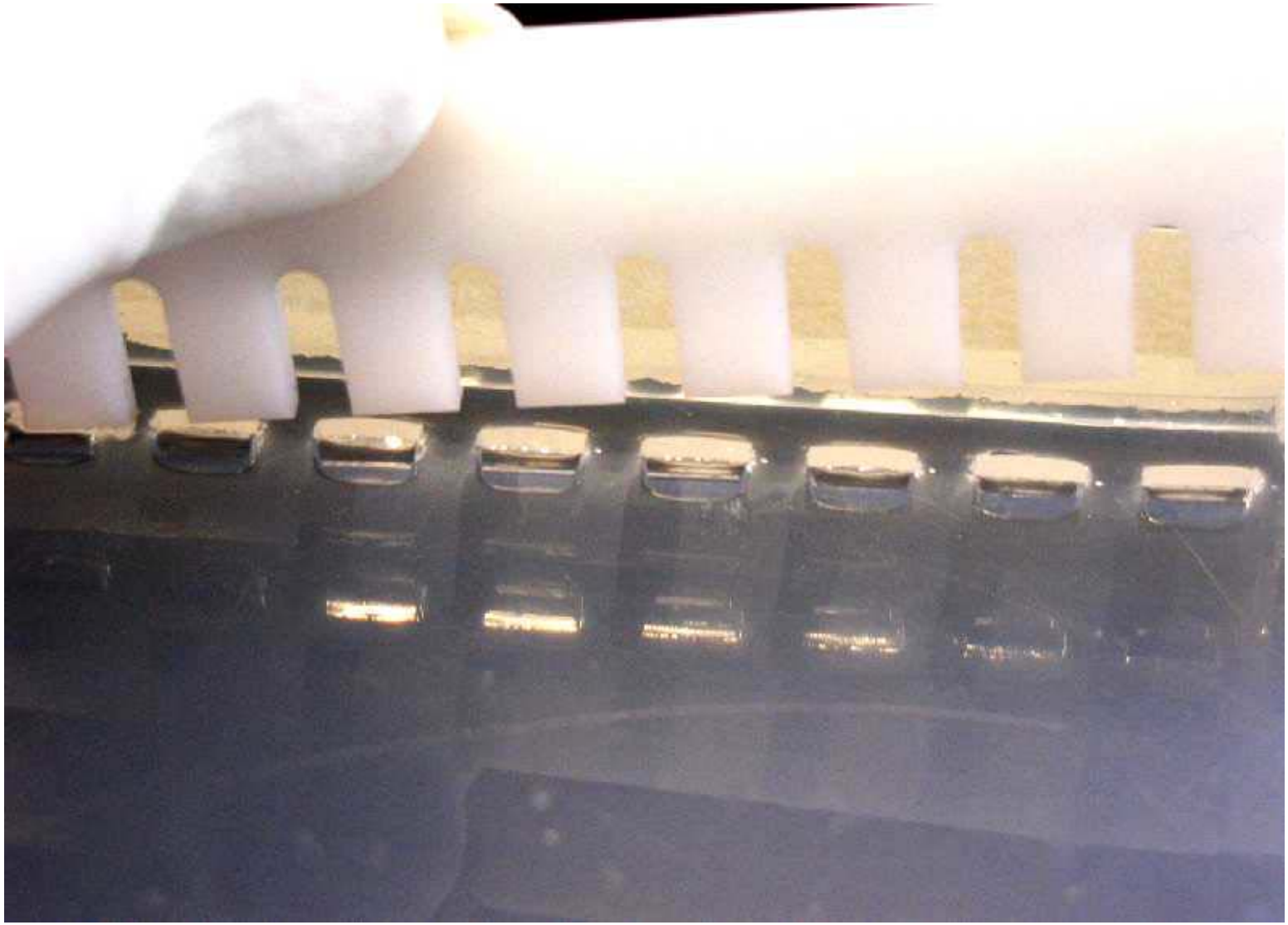
- Pouring
- Casting
- Loading
- Migration
- Running



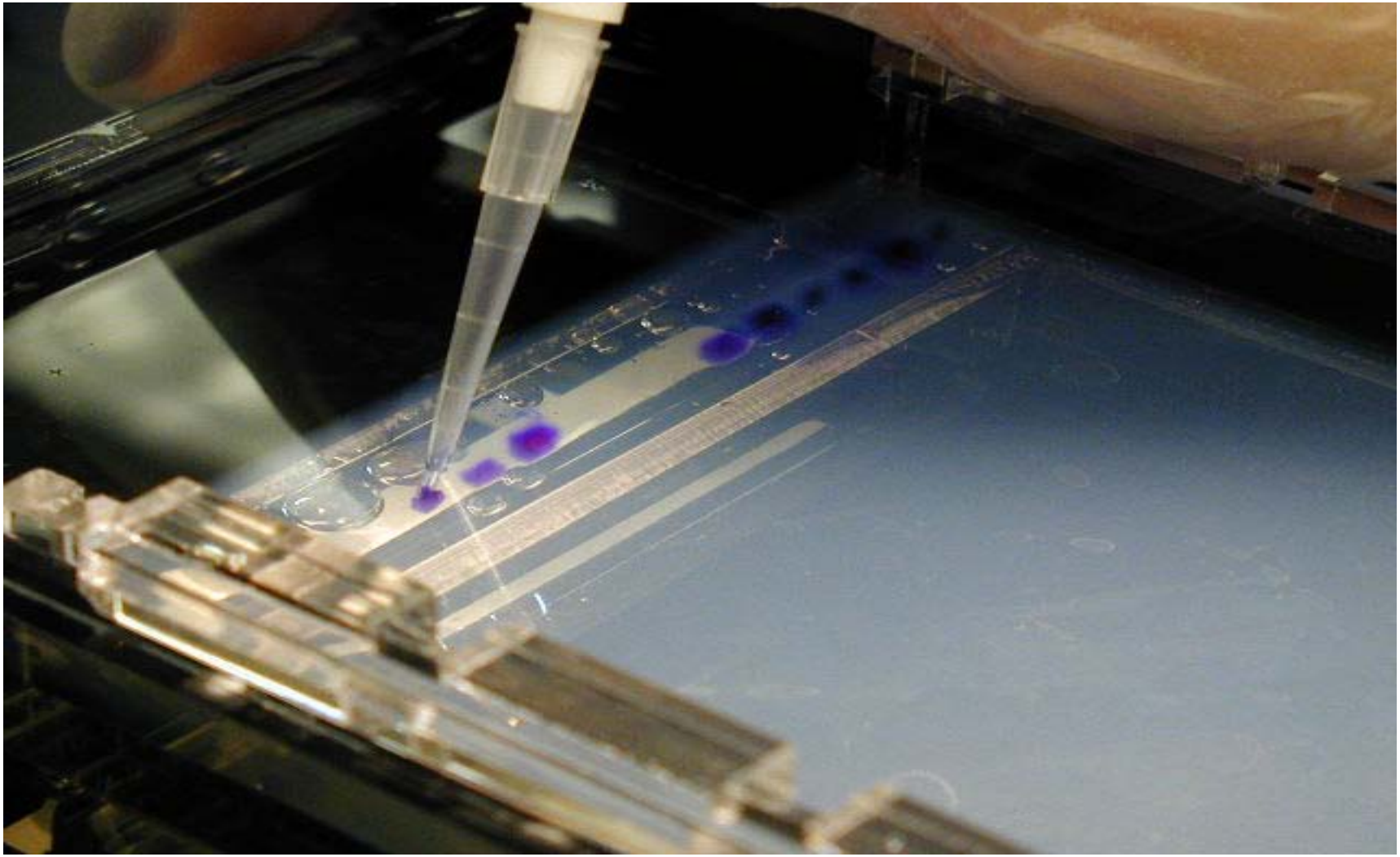




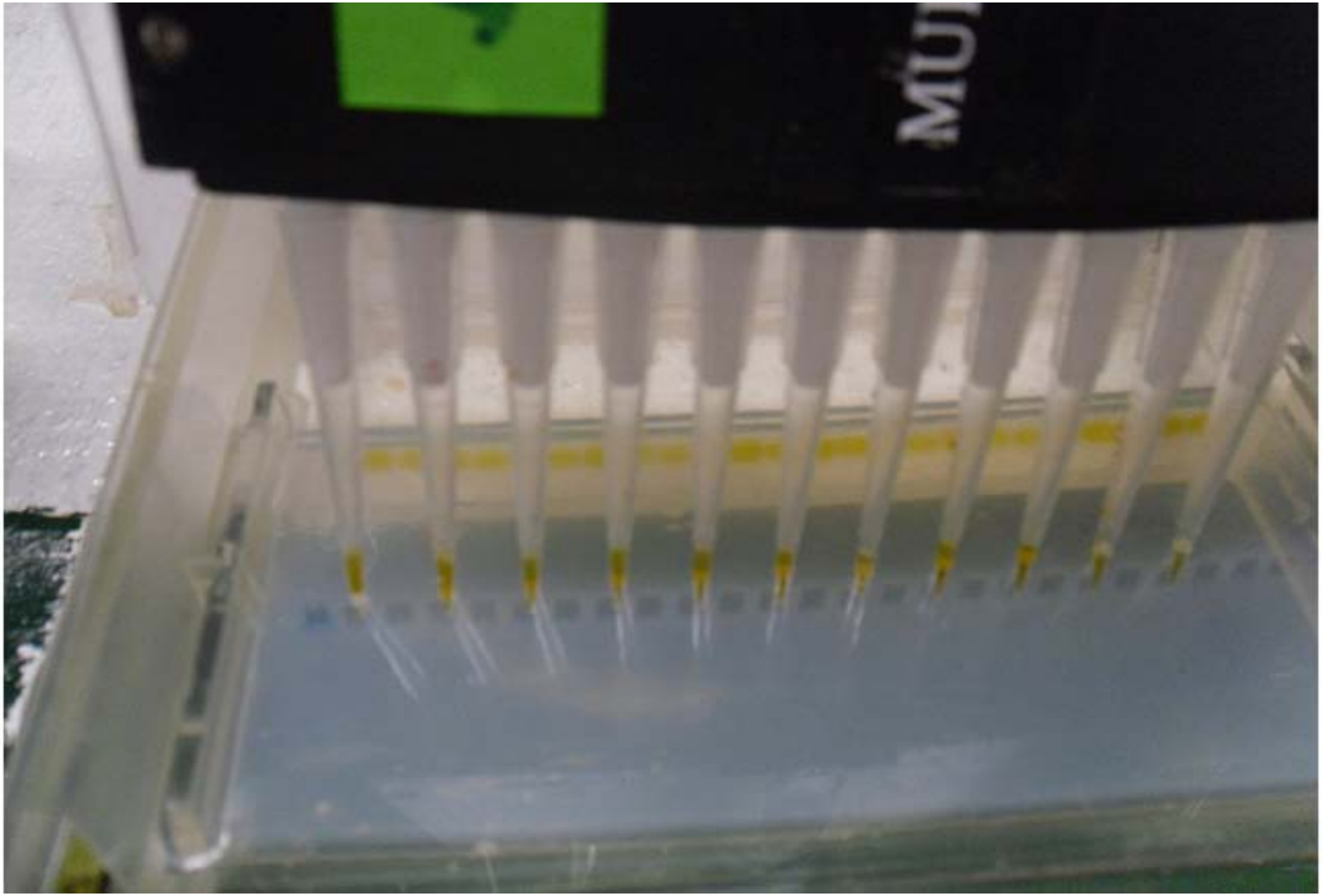
Sample wells



Sample loading

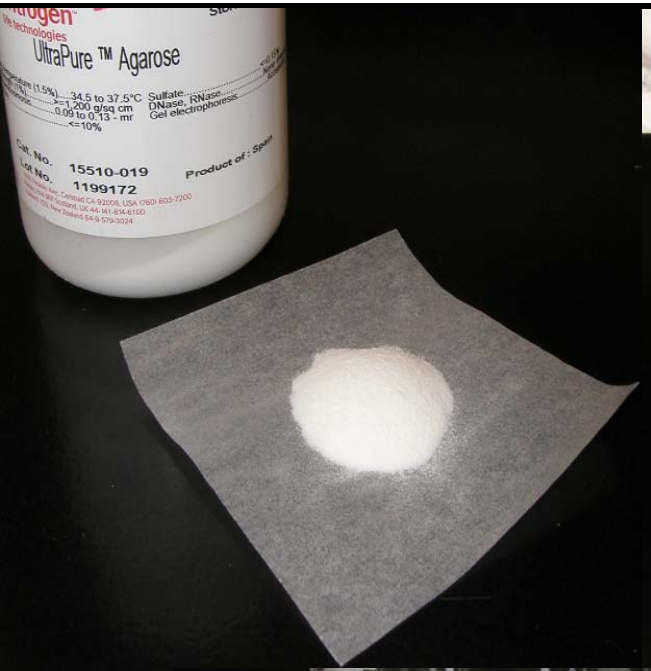


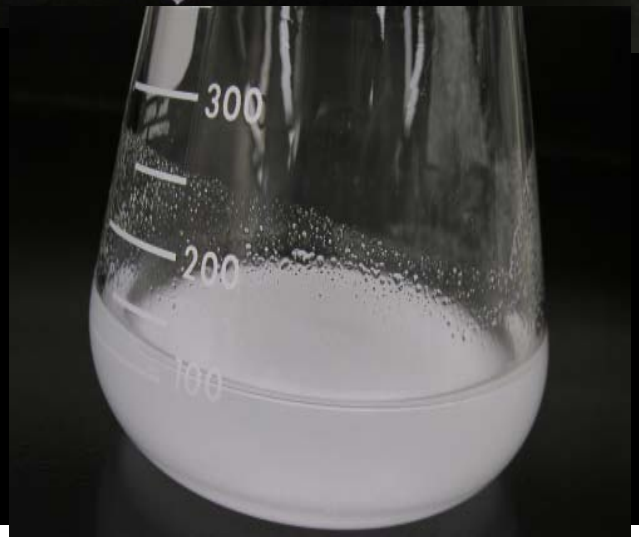
Loading with multi-channel pipette



Procedure
preparation electrophoresis
Experiment

1- Agarose Gel Preparing :





Before melting undissolved gel

after melting dissolved gel