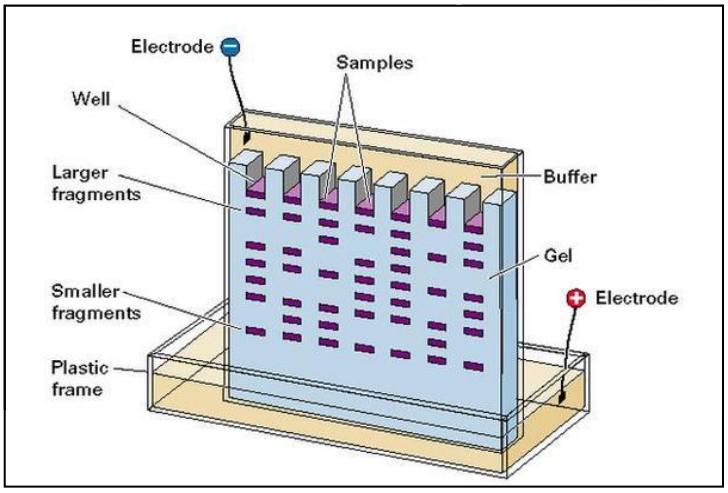
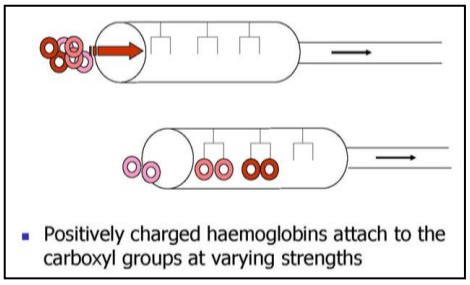
Problem 2 – Unit 6 – Biochemistry: Hb electrophoresis

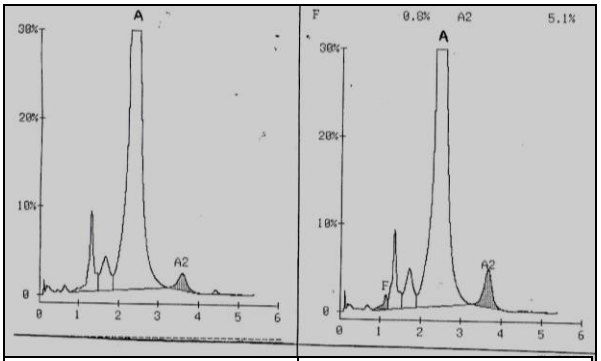
- **Principle of electrophoresis**: movement of charged molecules from cathode (-) to anode (+) in a gel under the influence of an electrical field.



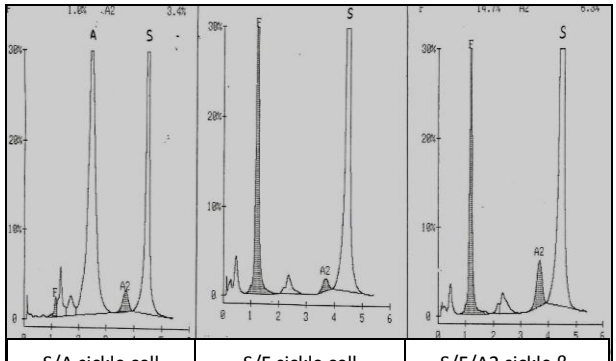
- **The rate of migration of the molecules is dependent on:**
  - Charge of the molecules.
  - Size.
  - Shape.
  - Buffer pH.
  - Time frame of the procedure.
  - Temperature of the operating system.
- **Protein electrophoresis:**
  - **More complex than DNA electrophoresis (WHY?)**
    - ✓ Different proteins have different charges.
    - ✓ Proteins vary widely in their shape.
  - **Gel medium**: polyacrylamide.
  - **Separation of proteins occur under:**
    - ✓ *Denaturing conditions*: in which proteins are treated with anionic detergents so they will maintain their charge but will lose their normal shape.
    - ✓ *Non-denaturing conditions*: in which there will be no pre-treatment of proteins prior to electrophoresis so they will retain both their charge and normal shape.
- **Hemoglobin electrophoresis:**
  - **Aim**: to diagnose hemoglobin pattern and any presence of abnormal hemoglobins.
  - **Medium**:
    - ✓ Cellulose acetate at alkaline pH (8.9).
    - ✓ Citrate agar at acidic pH (6).
  - Nowadays, it is widely replaced by HPLC.
- **HPLC:**
  - Positively charged molecules (salt and hemoglobin) will bind to negatively charged carboxyl groups (bound to silica).
  - Hemoglobin variants will separate out due to variation in charge.



HPLC vs. electrophoresis	
Advantages	**Disadvantages**
1. Automation: less time – more samples analyzed 2. Samples: very small (5µl). 3. Versatility: simultaneous quantification of HbA, HbA2, HbF, HbS and HbC. 4. Sensitivity: detecting δ chain in β-thalassaemia minor. 5. Quantitation.	1. Expensive initial setup. 2. Requires periodic optimization and skillful operation. 3. HbS co-elute with HbA2 rendering its quantification inaccurate.



Normal | A/A2 β-thalassemia trait



S/A sickle cell trait | S/F sickle cell disease | S/F/A2 sickle β-thalassemia