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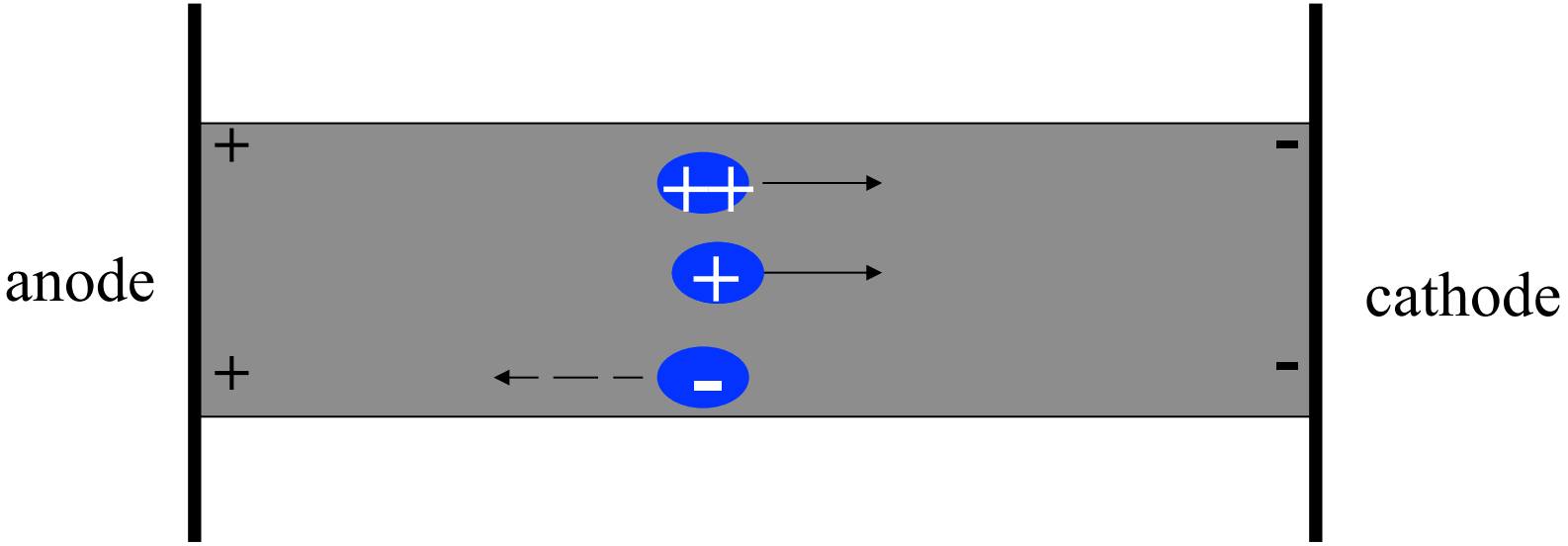
BCH 471
Experiment (11)

Separation of Serum Proteins by Cellulose Acetate Membrane Electrophoresis

Electrophoresis

- Its principle is that the charged particles of a sample migrate in an applied electrical field.
- It is applied for the separation and characterization of proteins, nucleic acids and subcellular-sized particles like viruses and small organelles.



Advantages of cellulose acetate membrane

- The virtual elimination of trailing because of the very small amount of adsorption.
- Well-defined bands are obtained on an almost colorless background making accurate quantitation possible.
- The volume of serum required is very small and with the small scale technique only a half to two hours is required for the separation.
- Subsequent staining, washing and drying is rapid so that the entire procedure usually takes no more 1-2 hours.

-Rate of migration in an electric field depends:

1- Net Charge

↑ net charge = ↑ migration

2- Size

↑ Size = ↓ migration

3-Electric field strength

↑ electric field strength = ↑ migration

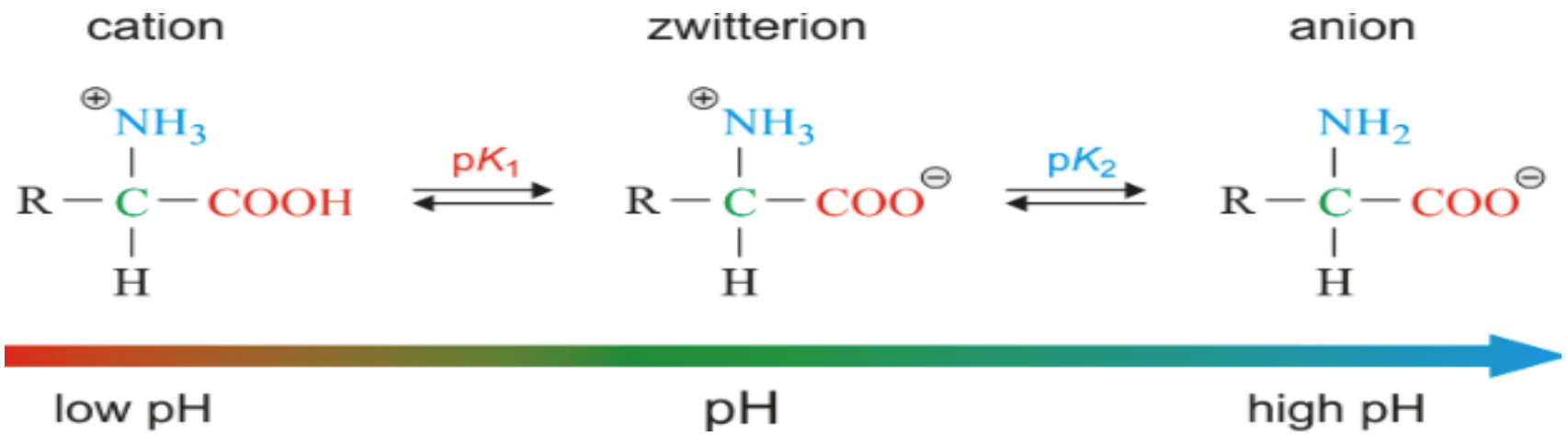
4- Temperature of operation

↑ Temp = ↑ migration

Effect of pH and buffer on protein charge

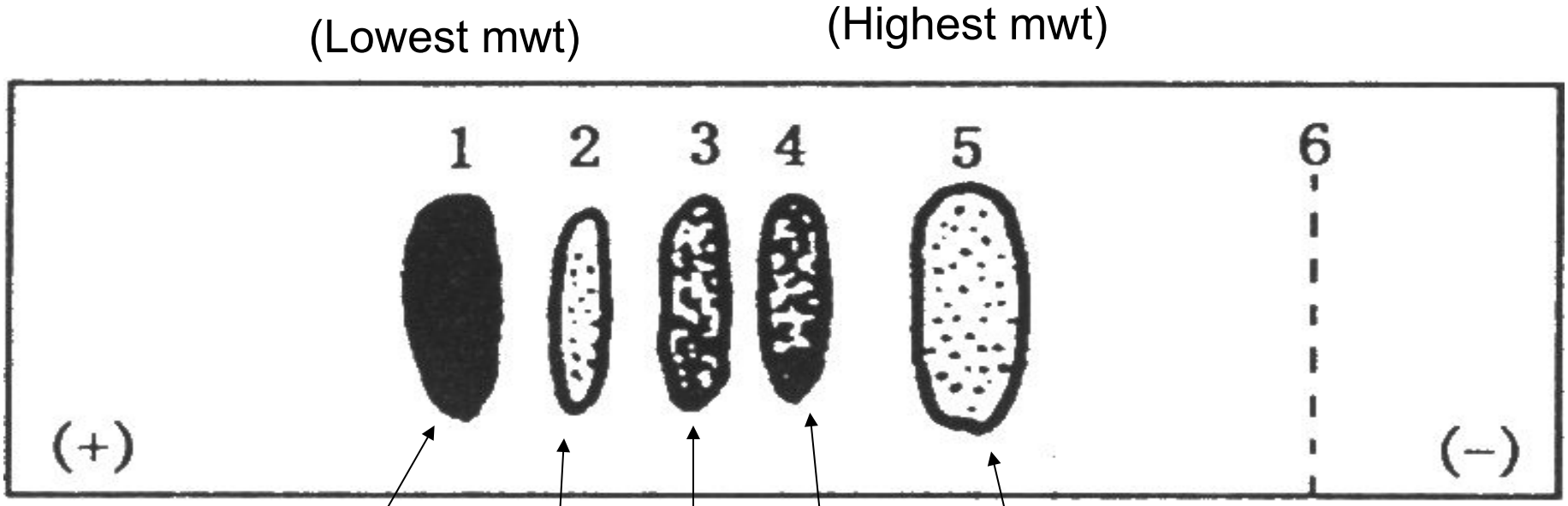
- Proteins are amphoteric compounds and are therefore either positively or negatively charged

- Isoelectric Point - pH where there is no net charge in molecule.



Barbitone buffer (pH 8.60, 0.06mol/L)

Albumin	**pI=4.9**
α1-globumin	**pI=5.28**
α2-globumin	**pI=5.82**
β-globumin	**pI=6.8**
γ-globumin	**pI=7.3**



1=albumin;
2, 3, 4, 5 is α1−, α2−, β− and γ−globumin;
6 is origine