Ministry of higher education and scientific research
AL-Mustaqbal University college
Department of medical physics

Analytical chemistry(practical)

Lecture 4

Chromatography
Electrophoresis Technique

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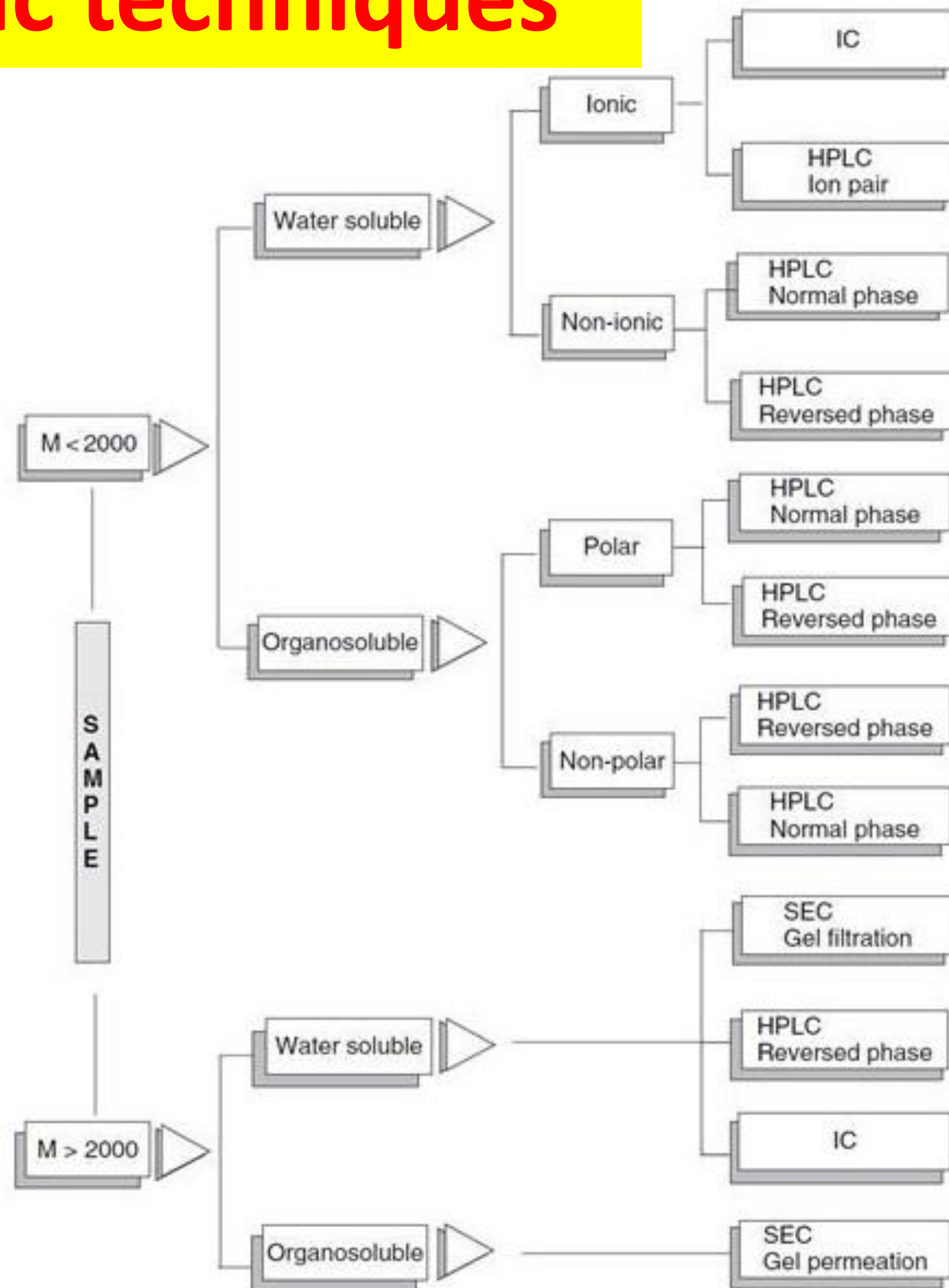
Chromatographic techniques

- ✓ This diagram represent Selection guide for all of the different chromatographic techniques with liquid mobile phases.
- ✓ The choice of technique is chosen as a function of the molar mass, solubility and the polarity of the compounds to be separated.



Introduction:

- ✓ Electrophoretic techniques are generally used for separation of charged analytes.
- ✓ Charged analytes move in electrolyte solutions when an electrical field is established.
- ✓ Separation is obtained if the charged analytes have different migration velocity.
- ✓ The electrolyte solution is most commonly a mixture of weak acids and bases in water.
- ✓ Electrophoretic techniques are widely used in biochemistry, especially for separation of nucleotides and proteins.

- ✓ The electrophoretic techniques can be divided into three main groups:

1. the traditional gel electrophoretic techniques .
2. the more recent capillary electrophoresis (CE).
3. potential-driven chromatography (electrochromatography) techniques.

- ✓ The term electrophoresis is used for the process where charged species (ions) in an electrolyte solution move under the influence of an electric field

Main Principle

- Electrophoretic separation is obtained if the charged analytes have different migration velocity u.
- An ion with the charge q will be subjected to a force F=q.E in an electric field, with field strength E=V/L, where V is the potential applied and L is the distance between the anode and the cathode ,see this figure 1 below

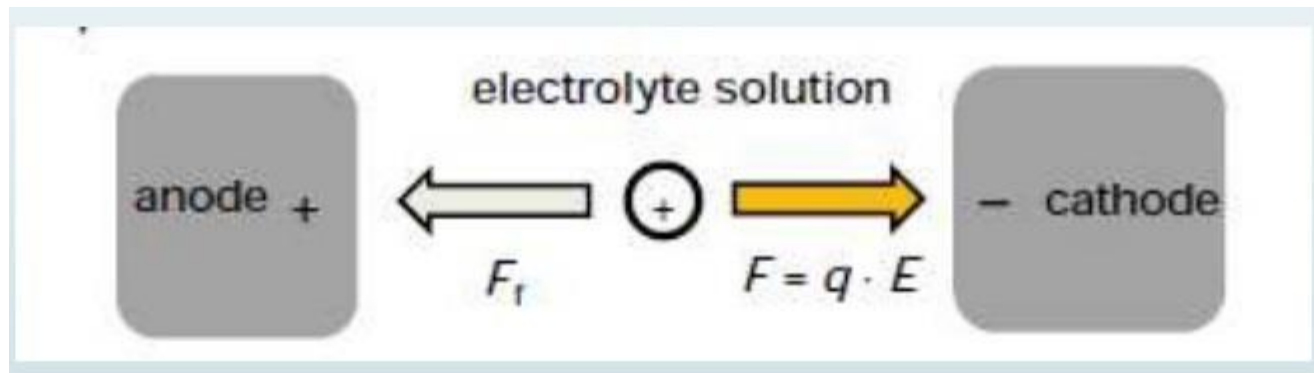


Figure: 1. Forces acting on a charged species in an applied electric field.

Due to the force, the ion will be accelerated for a very short time until the frictional force from the electrolyte solution Ff is equal to F. When Ff=F, the ion will move (migrate) at a constant velocity in the electrolyte solution.

- ✓ The frictional force **Ff** is given by

Stokes' law =Ff = 6 ηru

- ✓ Where η is the viscosity of the electrolyte solution and r is the radius of the ion.
- ✓ When **F=Ff**, the migration speed u can be found by:

U =qE/ 6 r η

This equation shows that the migration velocity **u .**

- increases with increasing ion charge q,
- decreases with increasing ion radius r,
- increases with increasing field strength E
- decreases with increasing temperature, since the viscosity **η** decreases

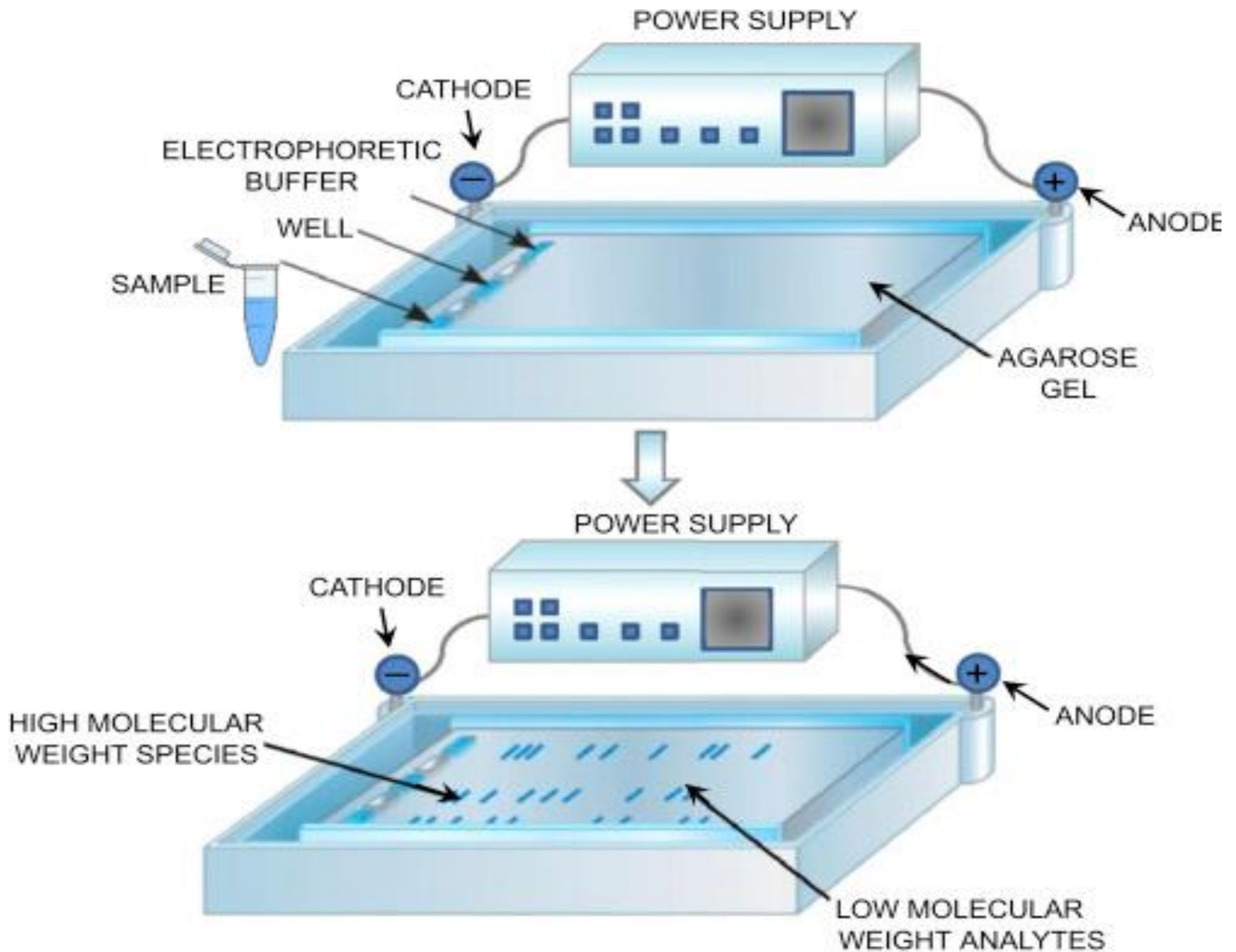
Gel Electrophoresis Techniques:

In electrophoretic separation techniques, as opposed to the chromatographic techniques, there is no mobile phase or stationary phase.

- ✓ In zone electrophoresis (a kinetic process), the migration length ($L=u.t$) is proportional to the applied voltage ($u= \mu E$) and time, and separation of analytes with different charge/size ratio (q/r) is obtained.

μ =electrophoretic mobility

- ✓ Electrophoretic separation can also be carried out using techniques isoelectric focusing (IEF) (equilibrium process).
- ✓ Zone electrophoresis and isoelectric focusing are the techniques mostly used for analytical separations.


POWER SUPPLY
CATHODE
ELECTROPHORETIC
BUFFER
WELL
SAMPLE
ANODE
AGAROSE
GEL
POWER SUPPLY
CATHODE
ANODE
HIGH MOLECULAR
WEIGHT SPECIES
LOW MOLECULAR
WEIGHT ANALYTES

So ..What is gel electrophoresis?

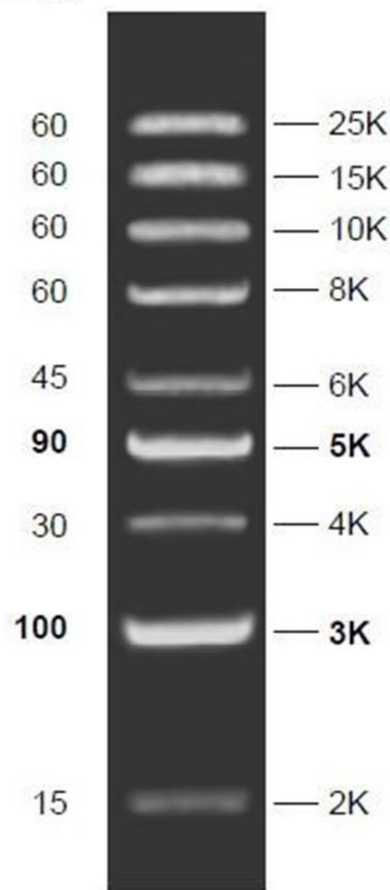
Gel electrophoresis is a technique commonly used in laboratories to separate charged molecules like DNA, RNA and proteins according to their size.

- ✓ Charged molecules move through a gel when an electric current is passed across it.
- ✓ An electric current is applied across the gel so that one end of the gel has a positive charge and the other end has a negative charge .
- ✓ The movement of charged molecules is called migration.
- ✓ Molecules migrate towards the opposite charge. A molecule with a negative charge will therefore be pulled towards the positive end vise versa .

- ✓ The gel consists of a permeable matrix, a bit like a sieve, through which molecules can travel when an electric current is passed across it.
- ✓ Smaller molecules migrate through the gel more quickly and therefore travel further than larger fragments that migrate more slowly and therefore will travel a shorter distance.
- ✓ As a result the molecules are separated by size.

Separation of Molecules by Size

- Small molecules can navigate through the pores of the gel more easily than large molecules.
- Small molecules migrate farther in the gel than large molecules.



0.7% TAE agarose gel

How can Gel Electrophoresis Separate DNA , RNA or even Protiens ?

- ✓ Electrophoresis can be use to distinguish DNA fragments of different lengths.
- ✓ DNA is negatively charged, therefore, when an electric current is applied to the gel, DNA will migrate towards the positively charged electrode. Shorter strands of DNA move more quickly through the gel than longer strands resulting in the fragments being arranged in order of size.
- ✓ The use of dyes, fluorescent tags or radioactive labels enables the DNA on the gel to be seen after they have been separated. They will appear as bands on the gel.

- ✓ A DNA marker with fragments of known lengths is usually run through the gel at the same time as the samples.
- ✓ By comparing the bands of the DNA samples with those from the DNA marker, you can work out the approximate length of the DNA fragments in the samples.

How is gel electrophoresis carried out?

1- Preparing the gel

- ✓ Agarose gels are typically used to visualize fragments of DNA.
- ✓ The concentration of agarose used to make the gel depends on the size of the DNA fragments you are working with.
- ✓ The higher the agarose concentration, the denser the matrix and vice versa.
- ✓ Smaller fragments of DNA are separated on higher concentrations of agarose whilst larger molecules require a lower concentration of agarose.

1.To make a gel

agarose powder is mixed with an electrophoresis buffer and heated to a high temperature until all of the agarose powder has melted. The molten gel is then poured into a gel casting tray and a “comb” is placed at one end to make wells for the sample to be pipetted into.
Once the gel has cooled and solidified (it will now be opaque rather thanclear) the comb is removed.
Many people now use pre-made gels.
The gel is then placed into an electrophoresis tank and electrophoresis
buffer is poured into the tank until the surface of the gel is covered. Thebuffer conducts the electric current. The type of buffer used depends on the approximate size of the DNA fragments in the sample.

2.Preparing the DNA for electrophoresis

- ✓ A dye is added to the sample of DNA prior to electrophoresis to increase the viscosity of the sample which will prevent it from floating out of the wells and so that the migration of the sample through the gel can be seen.
- ✓ A DNA marker (also known as a size standard or a DNA ladder) is loaded into the first well of the gel. The fragments in the marker are of a known length so can be used to help approximate the size of the fragments in the samples.
- ✓ The prepared DNA samples are then pipetted into the remaining wells of the gel.
- ✓ When this is done the lid is placed on the electrophoresis tank making sure that the orientation of the gel and positive and negative electrodes is correct (we want the DNA to migrate across the gel to the positive end).

3.Separating the fragments

- ✓The electrical current is then turned on so that the negatively charged DNA moves through the gel towards the positive side of the gel.
- ✓Shorter lengths of DNA move faster than longer lengths so move further in the time the current is run.
- ✓The distance the DNA has migrated in the gel can be judged visually by monitoring the migration of the loading buffer dye.
- ✓The electrical current is left on long enough to ensure that the DNA fragments move far enough across the gel to separate them, but not so long that they run off the end of the gel.

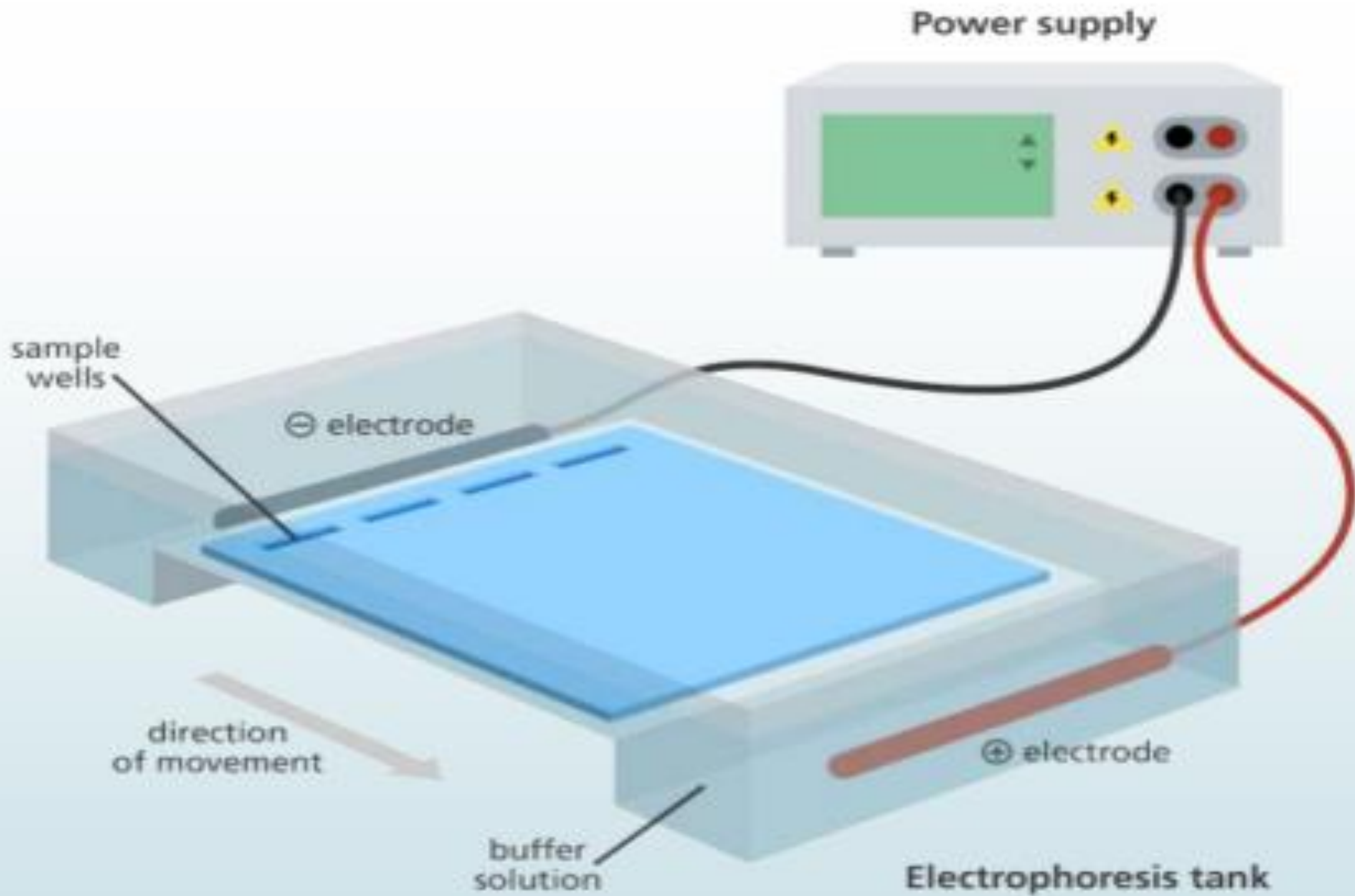
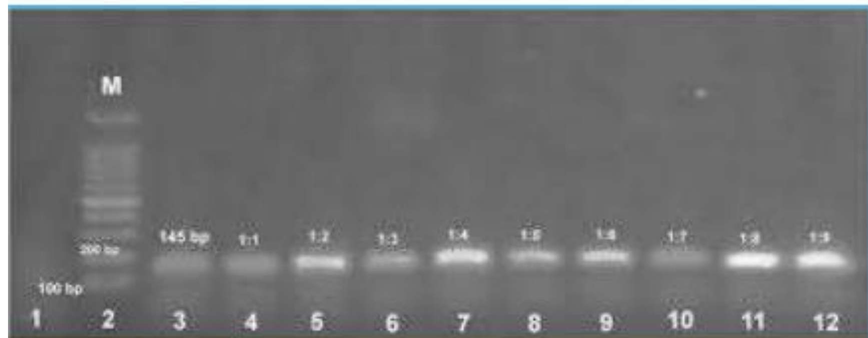
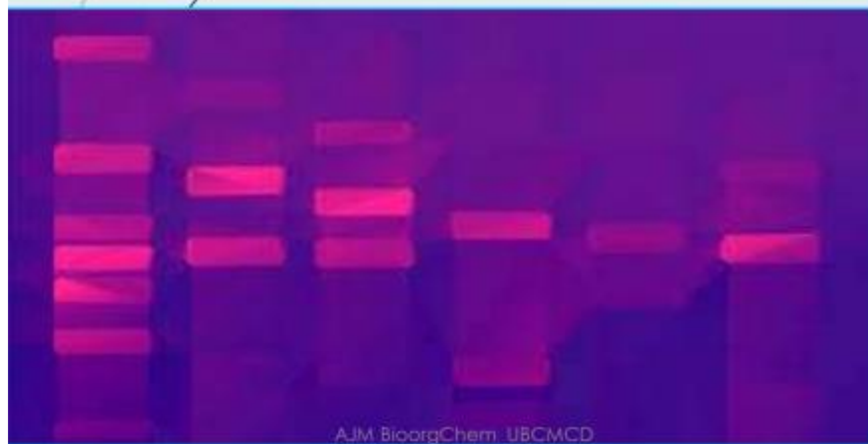


Figure shows: Illustration of DNA electrophoresis equipment used to separate DNA fragments by size. A gel sits within a tank of buffer. The DNA samples are placed in wells at one end of the gel and an electrical current passed across the gel. The negatively charged DNA moves towards the positive electrode

4.Visualizing the results

- ✓ Once the DNA has migrated far enough across the gel, the electrical current is switched off and the gel is removed from the electrophoresis tank.
- ✓ To visualize the DNA, the gel is stained with a fluorescent dye that binds to the DNA, and is placed on an ultraviolet transilluminator which will show up the stained DNA as bright bands.
- ✓ Alternatively the dye can be mixed with the gel before it is poured.
- ✓ If the gel has run correctly the banding pattern of the DNA marker/size standard will be visible.
- ✓ It is then possible to judge the size of the DNA in your sample by imagining a horizontal line running across from the bands of the DNA marker. You can then estimate the size of the DNA in the sample by matching them against the closest band in the marker.





samples

Marker	A	B	C

1,200 bp
1,000 bp
900 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp

Activa

How to separate Proteins?

We use polyacrylamide instead of agarose gel…. Next lecture

THANK YOU :)