10.4 VARIANTS OF THE GEL ELECTROPHORESIS

On the Basis of Substrate (AGE and PAGE)

(a) Agarose Gel Electrophoresis (AGE)

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA. This simple, but precise, analytical procedure is used in research, biomedical and forensic laboratories. It is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for determining the size of DNA molecules in the range of 500 to 30,000 base pairs. Nucleic acid molecules are size separated by the aid of an electric field where negatively charged molecules migrate toward anode (positive) pole. The migration flow is determined solely by the molecular weight where small weight molecules migrate faster than larger ones.

DNA gels are invariably run as horizontal, submarine or submerged gels; so named because such a gel is totally immersed in buffer. Agarose, dissolved in gel buffer by boiling, is poured onto a glass or plastic plate, surrounded by a wall of adhesive tape or a plastic frame to provide a gel about 3mm in depth. Loading wells are formed by placing a plastic well-forming template or comb in the poured gel solution, and removing this comb once the gel has set. The gel is placed in the electrophoresis tank, covered with buffer, and samples loaded by directly injecting the sample into the wells. Samples are prepared by dissolving them in a buffer solution that contains sucrose, glycerol or Ficoll, which makes the solution dense and allows it to sink to the bottom of the well. A dye such as bromophenol blue is also included in the sample solvent; it makes it easier to see the sample that is being loaded and also acts as a marker of the electrophoresis front. No stacking gel is needed for the electrophoresis of DNA because the mobilities of DNA molecules are much greater in the well than in the gel, and therefore all the molecules in the well pile up against the gel within a few minutes of the current being turned on, forming a tight band at the start of the run. General purpose gels are approximately 25 cm long and 12 cm wide. A higher voltage would cause excessive heating. For rapid analyses that do not need extensive separation of DNA molecules, it is common to use mini-gels that are less than 10 cm long.

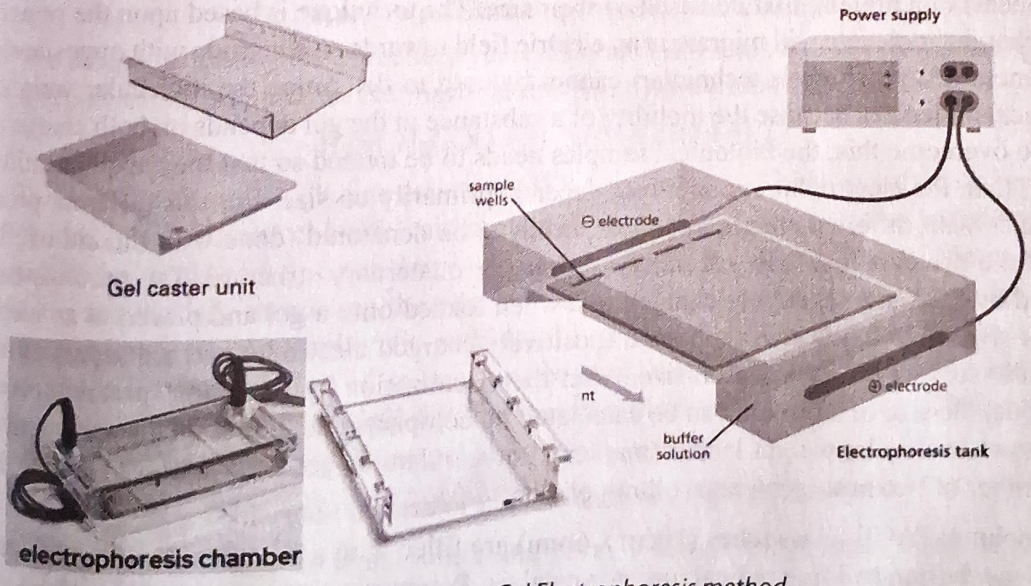

Fig. 10.5. Agarose Gel Electrophoresis method.

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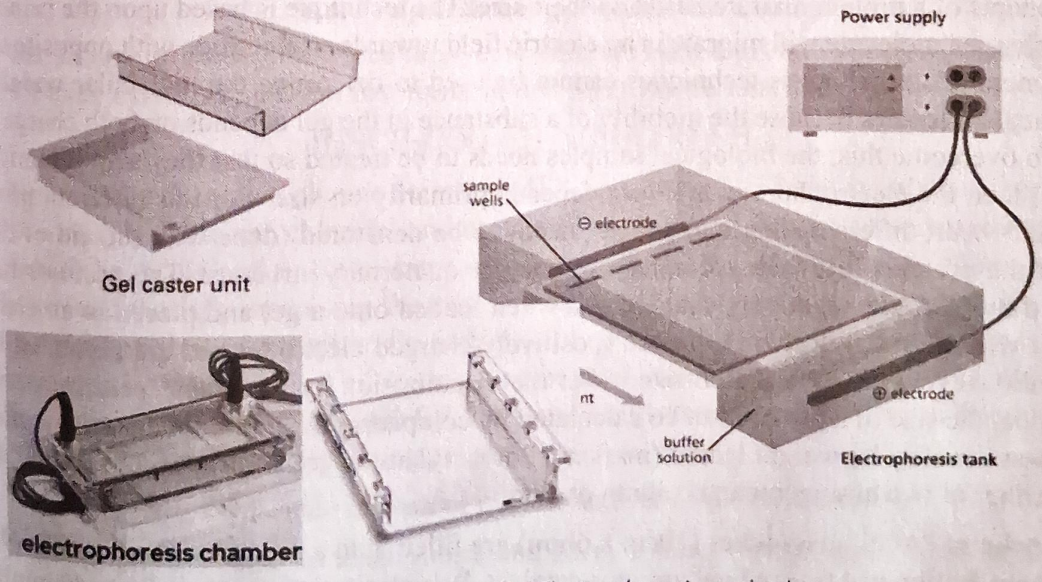

Fig. 10.5. Agarose Gel Electrophoresis method.

Once the system has been run, the DNA in the gel needs to be stained and visualised reagent most widely used is the fluorescent dye ethidium bromide. The gel is rinsed ge a solution of ethidium bromide (0.5 mg cm_3) and then viewed under ultraviolet light (3 wavelength). Ethidium bromide is a cyclic planar molecule that binds between the st base-pairs of DNA (i.e. it intercalates). The ethidium bromide concentration therefore bui at the site of the DNA bands and under ultraviolet light

Factors affecting migration of DNA in agarose gels.

1. Size of the DNA (length in base pairs, bp, or kilobase pairs, kb): large DNAs mi slowly (because it is more difficult for them to wiggle through the pores), whereas sm DNAs migrate faster (because they can easily penetrate the pores; you can think of the ge a "sieve").

2. Agarose concentration: the lower the concentration, the faster the DNA fragments migrate.

3. Conformation of the DNA: the relationship between rate of migration and size of DNA explained above only applies to linear DNA; different forms of circular DNA, e undigested plasmid DNA, have different properties and will migrate with different rates as same DNAs in their linear form; plasmids and other circular DNAs must therefore be linearis by restriction digestion before running them on a gel with the goal to determine the size of DNA.

4. Voltage applied: the higher the voltage, the faster the DNA will migrate; do not exce 5-8 V/ cm; it is best to check the current and make sure that it not exceeds 75 mA for standa size gels and 100 mA for minigels; heat generated at higher voltages/currents will impair resolution or even melt the gel.

5. Buffer composition: the salt content (ionic strength) of the electrophoresis buffer w influence migration; without salt the DNA will barely move; in the presence of too much sa the conductivity will be very high and the produced heat will impair separation.

(b) Polyacrylamide Gel Electrophoresis (PAGE)

PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separa components of a protein mixture based on their size. The technique is based upon the princip that a charged molecule will migrate in an electric field towards an electrode with opposite sig The general electrophoresis techniques cannot be used to determine the molecular weight biological molecules because the mobility of a substance in the gel depends on both charge an size. To overcome this, the biological samples needs to be treated so that they acquire unifor charge, then the electrophoretic mobility depends primarily on size. For this different protei molecules with different shapes and sizes, needs to be denatured (done with the aid of SDS so that the proteins lost their secondary, tertiary or quaternary structure .The proteins bein covered by SDS are negatively charged and when loaded onto a gel and placed in an electri field, it will migrate towards the anode (positively charged electrode) and are separated by molecular sieving effect based on size. After the visualization by a staining (protein-specific technique, the size of a protein can be calculated by comparing its migration distance with tho of a known molecular weight ladder (marker). Polyacrylamide gel electrophoresis can be done using either of two arrangements, column or slab.

In column PAGE, glass tubes (10cm x 6mm) are filled with a mixture of acrylamide, N, N methylene, buffer, and free radical initiator-catalyst. Polymerization occurs in 30 to 40 minutes The gel column is inserted between two separate buffer reservoirs. The upper reservoir usually

contains the cathode and the lower the anode. Gel electrophoresis is usually carried out at basic pH, where most biological polymers are anionic; hence, they move down toward the anode. The sample to be analyzed is layered on top of the gel and voltage is applied to the system. A "tracking dye" is also applied, which moves more rapidly through the gel than the sample components. When the dye band has moved to the opposite end of the column, the voltage is turned off and the gel is removed from the column and stained with a dye.

Slab gels are now more widely used than column gels. A slab gel on which several samples may be analyzed is more convenient to make and use than several individual column gels. Slab gels also offer the advantage that all samples are analyzed in a matrix environment that is identical in composition. A plastic "comb" inserted into the top of the slab gel during polymerization forms indentations in the gel that serve as sample wells. Up to 20 sample wells may be formed. After polymerization, the comb is carefully removed and the wells are rinsed thoroughly with buffer to remove salts and any un-polymerized acrylamide. The gel plate is clamped into place between two buffer reservoirs, a sample is loaded into each well, and voltage is applied. For visualization, the slab is removed and stained with an appropriate dye.

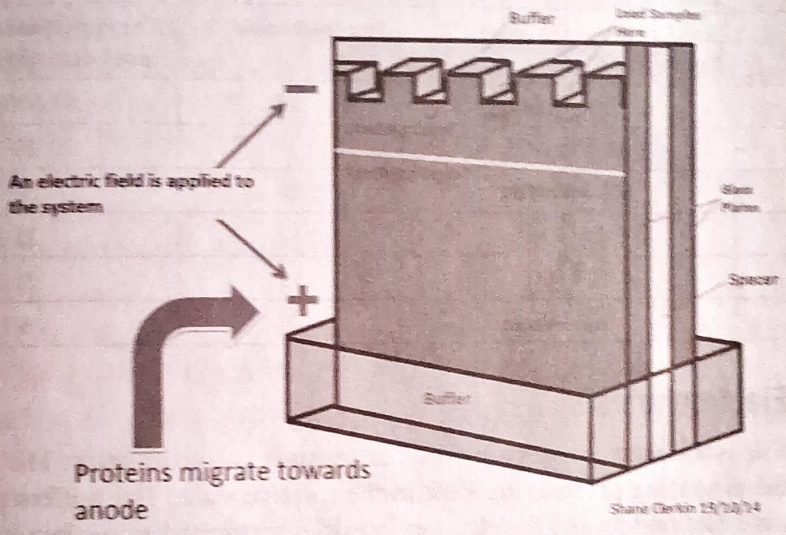

Fig. 10.6. *Polyacrylamide gel electrophoresis method.*

The sample is usually dissolved in glycine-chloride buffer, pH 8 to 9, before loading on the gel. Glycine exists primarily in two forms at this pH, a zwitterions and an anion.

$$H_3NCH_2COO = H_2NCH_2COO + H_2.$$

The average charge on glycine anions at pH 8.5 is about -2. When the voltage is turned on, buffer ions (glycinate and chloride) and protein or nucleic acid sample move into the stacking gel, which has a pH of 6.9. Upon entry into the upper gel, the equilibrium of above equation shifts toward the left, increasing the concentration of glycine zwitterions, which has no net charge and hence no electrophoretic mobility. In order to maintain a constant current in the electrophoresis system, a flow of anions must be maintained. Since most proteins and nucleic acid samples are still anionic at pH 6.9, they replace glycinate as mobile ions. Therefore, the relative ion mobilities in the stacking gel are:

chloride> protein or nucleic acid sample> glycinate.

The sample will tend to accumulate and form a thin, concentrated band sandwiched between the chloride and glycinate as they move through the upper gel. Since the acrylamide

concentration in the stacking gel is low (2 to 3%), there is little impediment to the mobility of the large sample molecules.

Now, when the ionic front reaches the lower gel with pH 8 to 9 buffer, the glycinate concentration increases and anionic glycine and chloride carry most of the current. The protein or nucleic acid sample molecules, now in a narrow band, encounter both an increase in pH and a decrease in pore size. The increase in pH would, of course, tend to increase electrophoretic movement of anions in the lower gel is

chloride> glycinate> protein or nucleic acid sample.

The separation of sample components in the resolving gel occurs in the similar way. Each component has a unique charge/mass ratio and a discrete size and shape, which directly influence its mobility.

TABLE 10.1 *Relative percentage of gels prepared from agarose or gel electrophoresis and the size range of DNA suitable for particular gel. [Source: https://www.idtdna.com/].*

Agarose Gels		*Polyacrylamide Gels*	
% agarose	*Size Range for Optimum Resolution (bp)*	*% acrylamide*	*Size Range for Optimum Resolution (bp)*
0.5	1,000-30,000	3.5	1,000-2,000
0.7	800-12,000	5	80-500
1.0	500-10,000	8	60-400
1.2	400-700	12	25-150
1.5	200-500	15	25-150
		20	6-100