Medical Laboratory Techniques Department

Lab. 6: Gel Electrophoresis and Gel documentation system

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Introduction

Electrophoresis is a technique used for sorting of macromolecules (Nucleic acid and Proteins) based on size and charge through a specific medium (agarose gel or polyacrylamide gel) that occurs when an electric field applied .

• The molecules (Nucleic acid and Proteins') to be separated are pushed by an electrical field through a gel that contains small pores . The molecules travel through the pores in the gel at a speed that is inversely related to their lengths

•The gel is placed in an electrophoresis chamber, which is then connected to a power source..

•The electric field consists of a negative charge at one end which pushes the molecules through the gel and a positive charge at the other end that pulls the molecules through the gel.

• Positively charged (cations) will migrate towards the (cathode) which is negatively charged.

• Negatively charged (anions) they will migrate towards the positively charged anode.

• The larger molecules move more slowly through the gel while the smaller molecules move faster.

• The different sized molecules form distinct bands on the gel.

• If several samples have been loaded into adjacent wells in the gel, they will run parallel in individual lanes.

It is used in

1. clinical chemistry to separate proteins by charge and/or size.

2. Biochemistry and Molecular biology to separate DNA and RNA fragments by length, or to separate proteins by charge.

Types of gel electrophoresis

Different types of gels which can be used are; Agar and Agarose gel, Starch, Sephadex , Polyacrylamide gels .

Agarose gel electrophoresis

Agarose is a polysaccharide extracted from seaweed. It is typically used at concentrations of 0.5 to 2%. The higher the agarose concentration the "stiffer" the gel . Higher percentages requiring longer run times.

It is a simple, cheap and Highly effective method for separating, identifying, And purifying DNA fragments because the pores of an agarose gel are large, so it is used to separate macromolecules such as nucleic acids (DNA), large proteins and protein complexes. . Agarose is in powdered form, and is insoluble in water at room temperature. It gets dissolved in boiling water and when it starts to cool, it undergoes cross-linking (H-bonding) and results in polymerization (agarose gel matrix).

DNA fragments of length ranging from 50 base pair to several million base pair can be separated using agarose gel electrophoresis. Migration rate of the fragments also depends on the concentration of agarose used to prepare gel. The concentration of agarose is inversely proportional to the rate of migration of the DNA fragments. Generally, used agarose concentration is 0.7% to separate DNA fragments of range 2- 10 kb and 2% agarose for separation of small fragments such as 0.1- 1 kb.

Preparation of the Gel

1 -Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.

2 -Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

3 -Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

4- Allow the agarose to cool either on the bench top or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray.

5- Add ethidium bromide (EtBr) to a concentration of 0.5 μg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 μg/ml EtBr for 15-30 min, followed by de-staining in running buffer for an equal length of time.

Note: EtBr is a suspected carcinogen and must be properly disposed of per institution regulations. Gloves should always be worn when handling gels containing EtBr. Alternative dyes for the staining of DNA are available; however EtBr remains the most popular one due to its sensitivity and cost.

6- Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.

7- Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to

"fall" into the sample wells, and one or two tracking dyes (Bromophenol blue , xylene cyanol FF, orange G, tartrazine) which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

8- Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use .

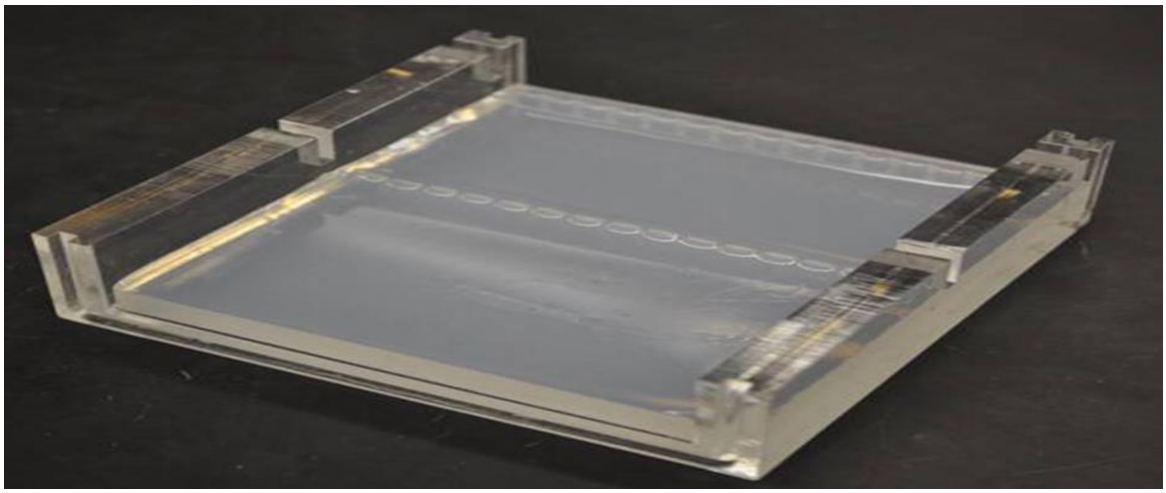
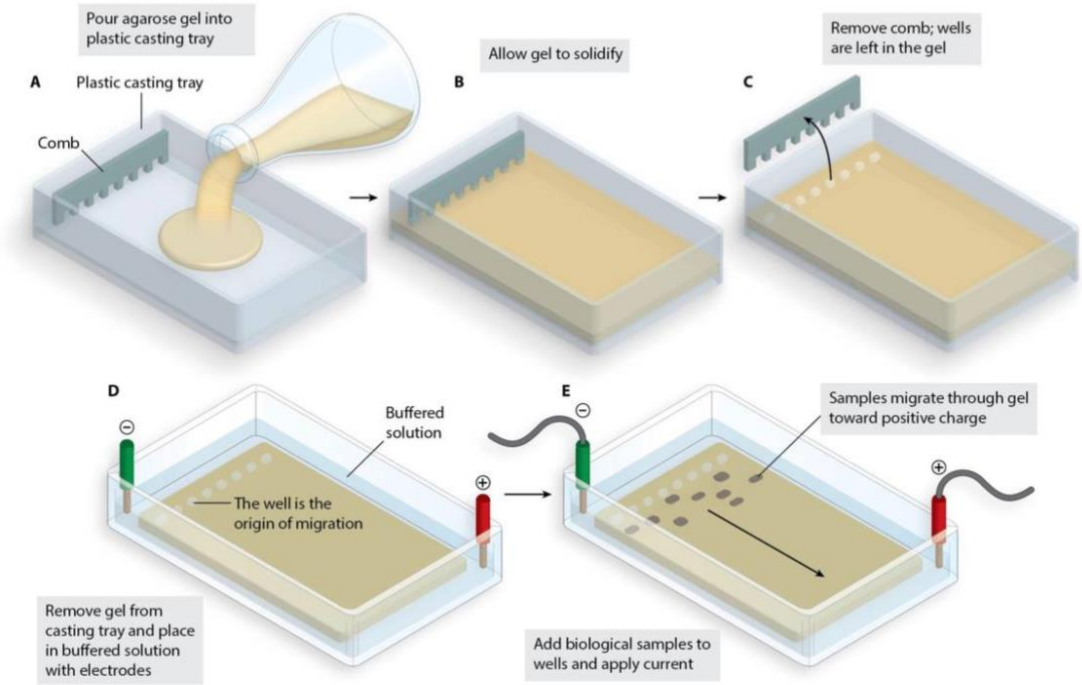


Figure : A solidified agarose gel after removal of the comb

Preparation of agarose:



Polyacrylamide gel electrophoresis

It is a subtype of the gel electrophoresis whereby the normal gel is replaced with polyacrylamide gels used as support media. Polyacrylamide is a cross-linked polymer of acrylamide. The length of the polymer chains is dictated by the concentration of acrylamide used, which is typically between 3.5 and 20%.

•It is used for separating proteins ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel. In contrast to agarose , polyacrylamide gels are used extensively for separating and characterizing mixtures of proteins.

Traditional DNA sequencing techniques used polyacrylamide gels to separate DNA fragments differing by a single base-pair in length so the sequence could be read. However, under appropriate conditions, fragments of DNA differing is length by a single base pair are easily resolved

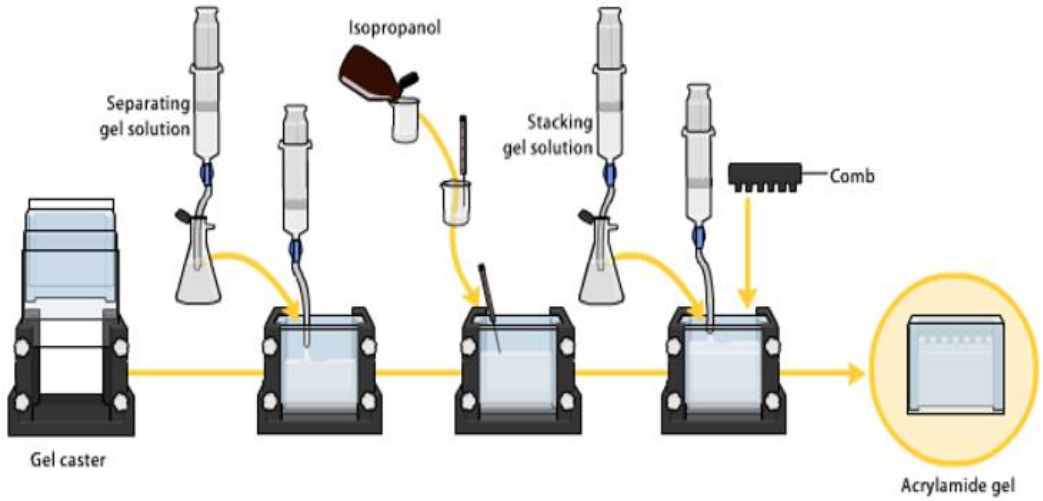
In the case of DNA, polyacrylamide is used for separating fragments of less than about 500 bp.

Preparation of Polyacrylamide

•Polyacrylamide gels are significantly more annoying to prepare than agarose gels.
•polyacrylamide forms in a chemical polymerization reaction.

•Oxygen inhibits the polymerization process, they must be poured between glass plates (or cylinders).

Acrylamide is a potent neurotoxin and should be handled with care! Wear disposable gloves when handling solutions of acrylamide, and a mask when weighing out powder. Polyacrylamide is considered to be non-toxic, but polyacrylamide gels should also be handled with gloves due to the possible presence of free acrylamide.



For prepaeration of 100 ml on TBE buffer

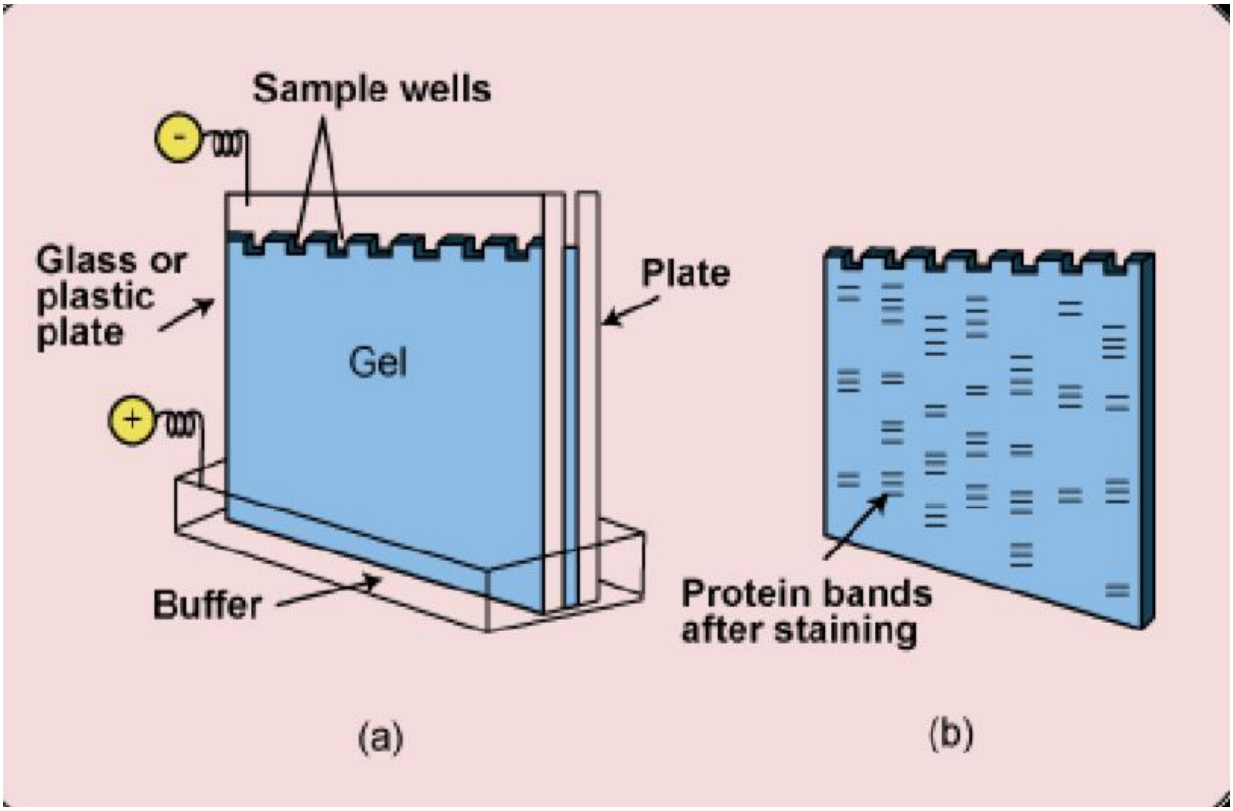


Figure (4) :- Polyacrylamide gel electrophoresis device

VISUALIZATION

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel.

Bands

• Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. This size usually measured by Markers.

Molecular weight size Marker/Ladder it is a substance that contains a mixture of molecules of known sizes. It runs on one lane parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size

Compare between agarose and polyacrylamide

Agarose gel	Polyacrylamide gel
Polysaccharide extracted from sea weed.	Cross-linked polymer of Acrylamide
Gel casted horizontally	Gel casted vertically
Non-toxic	Potent neuro-toxic
Separate large molecules	Separate small molecules
Commonly used for DNA separations	Used for DNA or protein Separations
Staining can be done before or pouring the gel.	Staining can be done after pouring the gel

Gel documentation

Gel documentation is a fundamental part of electrophoretic analysis. Cleaver Scientific provides a range of gel documentation systems suitable for both conventional ethidium bromide stained DNA, as well as modern ethidum bromide alternatives such as runSafe. Our omniDOC is a top of the range system taht can include blue, white and UV illumination. The compact microDOC system can be supplied with a UV transilluminator or blue light table and is equipped with a touch screen for PC free operation. The microDOC basic is the most economical option, providing excellent images at an affordable cost.