

## THEORY

### Principle of electrophoresis

If a mixture of electrically charged biomolecules is placed in an electric field of field strength that is they will freely move towards the electrode of opposite charge.

However, different molecules will move at quite different and individual rates depending on the physical characteristics of the molecule and on experimental system used.

$$V = \text{Drift velocity} \quad E = \text{Electric Field Strength}$$

$$F = \text{Frictional coefficient}$$

Describes frictional resistance to mobility and depends on a number of factors such as mass of the molecule, its degree of compactness, buffer viscosity and the porosity of the matrix in which the experiment is performed.

$$q = \text{Net charge}$$

The net charge is determined by the number of positive and negative charges in the molecule. Charges are conferred on proteins by amino acid side chains as well as by groups arising from post translational modifications such as deamidation, acylation or phosphorylation. DNA has a particularly uniform charge distribution since a phosphate group confers a single negative charge per nucleotide.

$$U_e = \frac{V}{E} = \frac{q}{F}$$

When a potential difference is applied, therefore, molecules with different overall charges will begin to separate owing to their different electrophoretic mobilities. Even molecules with similar charges will begin to separate if they have different molecular sizes, since they will experience different frictional forces. As will be seen below, some forms of electrophoresis rely almost totally on the different charges on molecules to effect separation, whilst other methods exploit differences in molecular size and therefore encourage frictional effects to bring about separation. Provided the electric field is removed before the molecules in the sample reach the electrodes, the components will have been separated according to their electrophoretic mobility. Electrophoresis is thus an incomplete form of electrolysis. The separated samples are then located by staining with an appropriate dye or by autoradiography if the sample is radiolabeled. The current in the solution between the electrodes is conducted mainly by the buffer ions, a small proportion being conducted by the sample ions.

Ohm's law expresses the relationship between current (I), voltage (V) and resistance (R)

$$V = iR$$

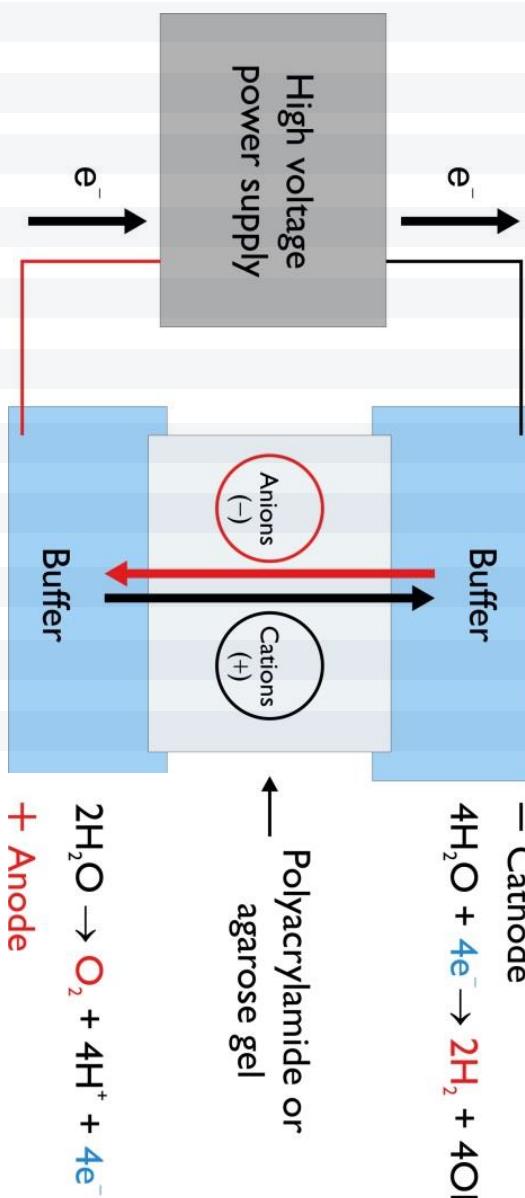
It therefore appears that it is possible to accelerate an electrophoretic separation by increasing the applied voltage, which would result in a corresponding increase in the current flowing. The distance migrated by the ions will be proportional to both current and time. However, this would ignore one of the major problems for most forms of electrophoresis, namely the generation of heat.

During electrophoresis the power (W, watts) generated in the supporting medium is given by

$$W = i^2 R$$

Most of this power generated is dissipated as heat. Heating of the electrophoretic medium has the following effects:

- An increased rate of diffusion of sample and buffer ions leading to broadening of the separated samples.
- The formation of convection currents, which leads to mixing of separated samples.
- Thermal instability of samples that are rather sensitive to heat. This may include denaturation of proteins (e.g. thus the loss of enzyme activity).
- A decrease of buffer viscosity, and hence a reduction in the resistance of the medium.

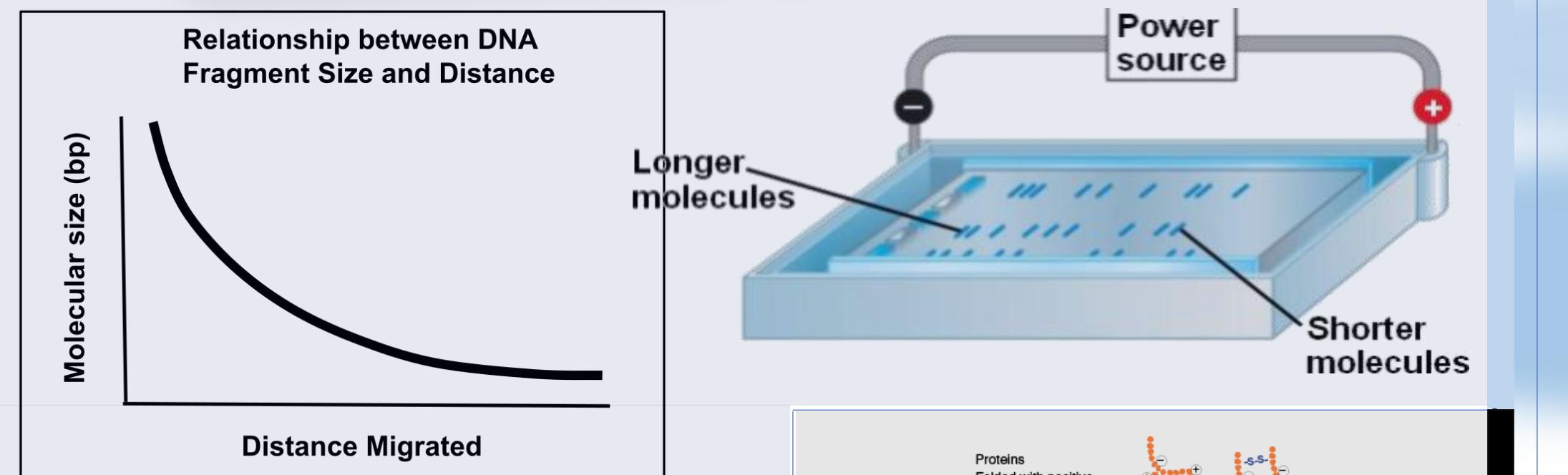
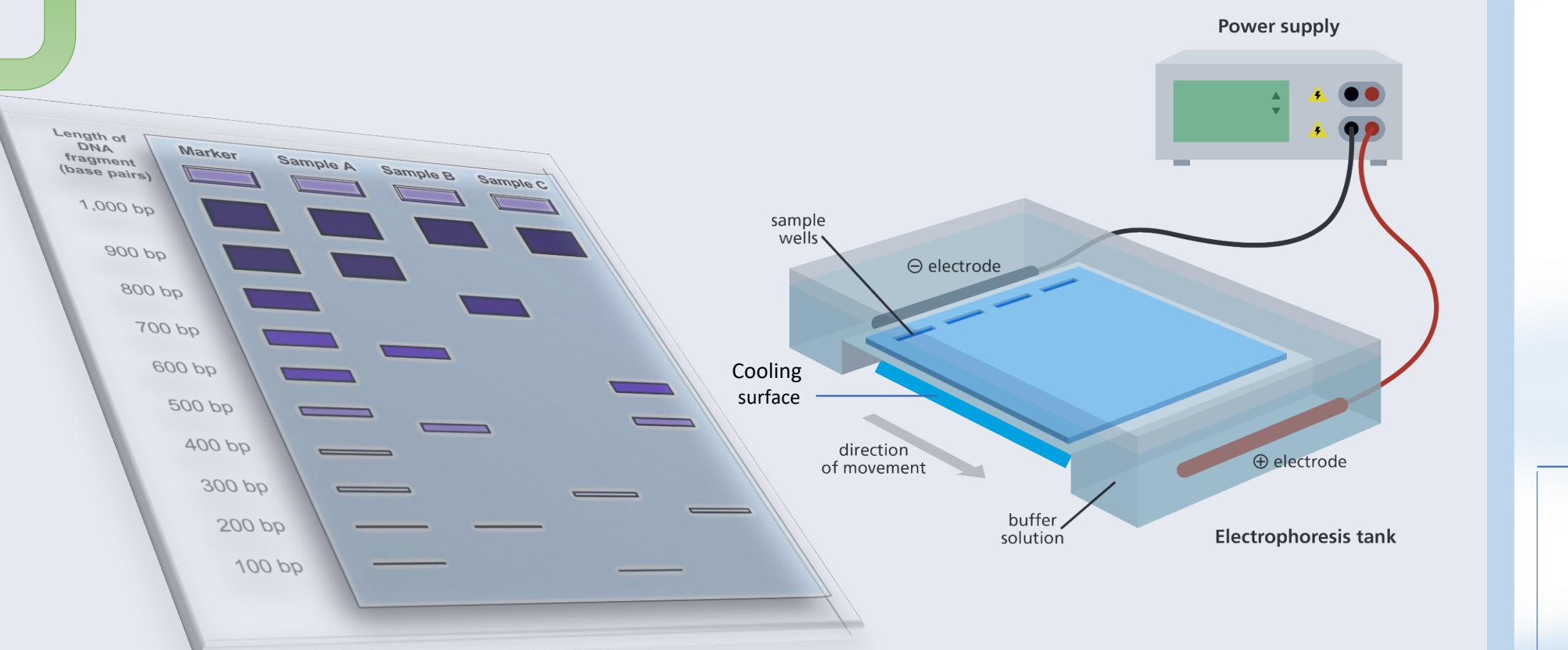
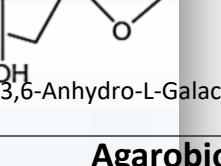
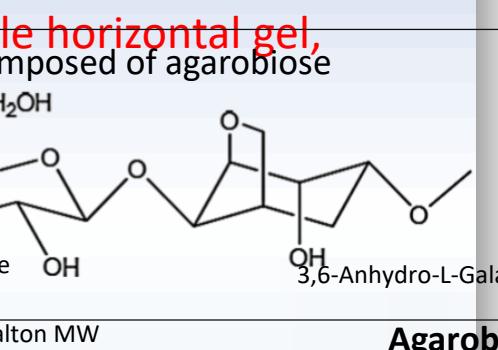


### Introduction

Positive or negative electrical charges are frequently associated with biomolecules. When placed in an electric field, charged biomolecules move towards the electrode of opposite charge due to the phenomenon of electrostatic attraction. Electrophoresis is the separation of charged molecules in an applied electric field. The relative mobility of individual molecules depends on several factors. The most important of which are net charge, charge/mass ratio, molecular shape and the temperature, porosity and viscosity of the matrix through which the molecule migrates.

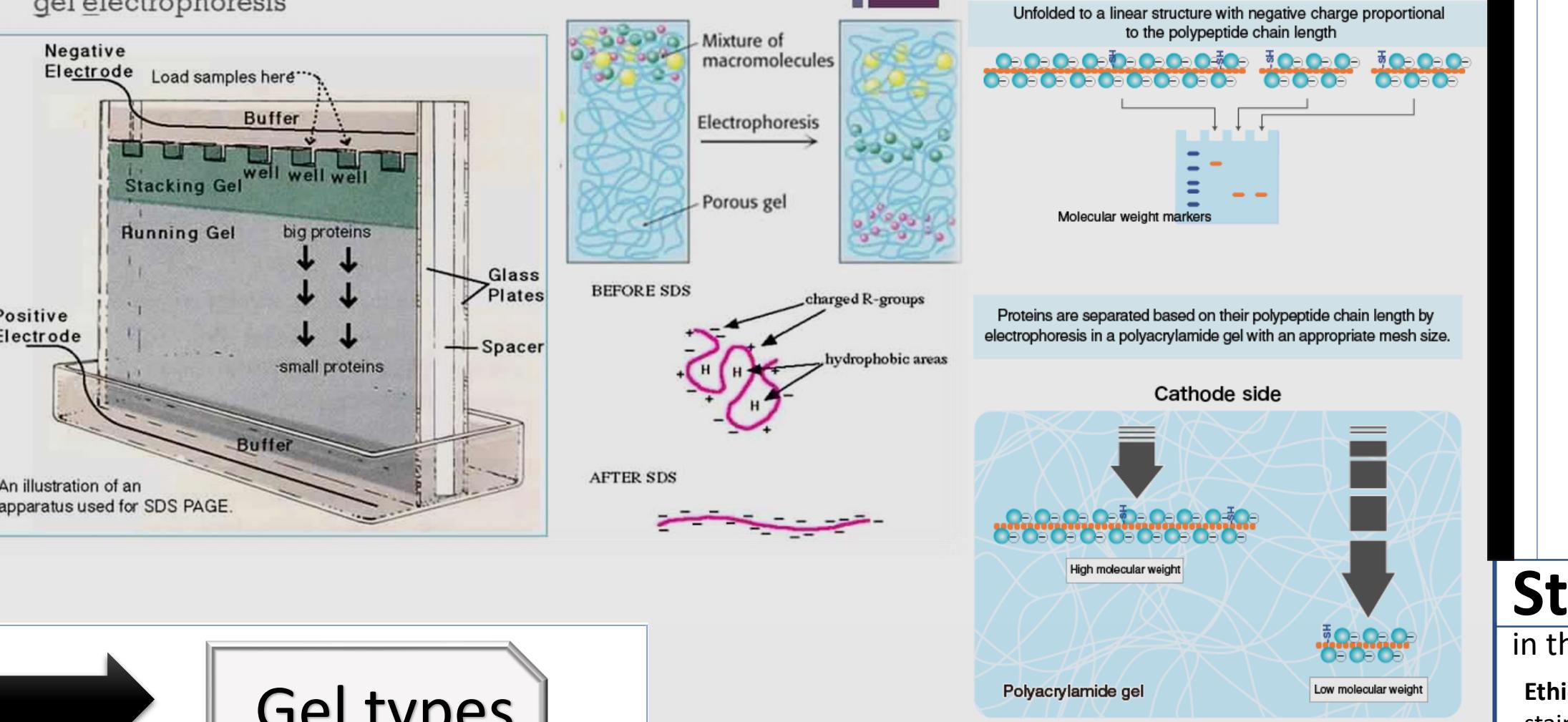
### Agarose

The most widely used polysaccharide gel matrix nowadays is that formed with agarose. This is a polymer composed of a repeating disaccharide unit called agarobiose which consists of galactose and 3,6-anhydrogalactose. Agarose gives a more uniform degree of porosity than starch and this may be varied by altering the starting concentration of the suspension (low concentrations give large pores while high concentrations give smaller pores). This gel has found wide spread use especially in the separation of DNA molecules (although it may also be used in some electrophoretic procedures involving protein samples such as immuno-electrophoresis). Because of the uniform charge distribution in nucleic acids, it is possible to accurately determine DNA molecular masses based on mobility in agarose gels. However the limited mechanical stability of agarose, while sufficient to form a stable horizontal gel, compromises the possibilities for post-electrophoretic manipulation.



### 1. Protein Electrophoresis

SDS-PAGE stands for sodium dodecyl sulfate polyacrylamide gel electrophoresis



### Gel types

In general the macromolecules solution is electrophoresed through some kind of matrix. The matrix acts as a molecular sieve to aid in the separation of molecules on the basis of size. The kind of supporting matrix used depends on the type of molecules to be separated and on the desired basis for separation: charge, molecular weight or both. The most commonly used materials for the separation of nucleic acids and proteins are agarose and acrylamide.

Medium	Conditions	Principal Uses
Starch	Cast in tubes or slabs	Proteins
Agarose gel	Cast in tubes or slabs	Very large proteins, nucleic acids
Acrylamide gel	Cast in tubes or slabs	Proteins and nucleic acids

### 3.3 Preparation and running of standard agarose gels

- The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:
- An electrophoresis chamber and power supply
- Gel casting trays, which are available in a variety of sizes and composed of Uvtransparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.

- Sample combs, around which molten medium is poured to form sample wells in the gel.
- Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- 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, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

• **Staining:** DNA molecules are easily visualized under an ultraviolet lamp when electrophoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When intercalated into double stranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate.

**NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.**

**NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.**

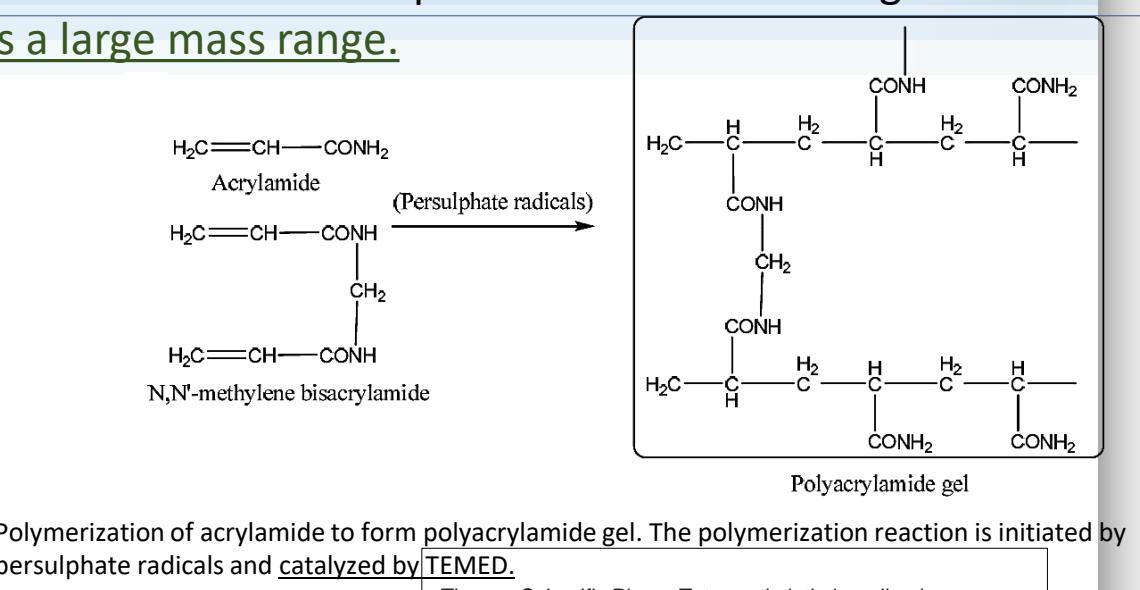
To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it. Ethidium bromide is added to the gel (final concentration 0.5  $\mu$ g/ml) to facilitate visualization of DNA after electrophoresis. After cooling the solution to about 60°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature. After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer solution. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus and a current is applied. The current flow can be confirmed by observing bubbles coming off the electrodes. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like Loading dye in our lab it is called (bromophenol blue and xylene cyanol dyes).

### Acrylamide

A far stronger gel suitable for electrophoretic separation of both proteins and nucleic acids may be formed by the polymerization of acrylamide. The inclusion of a small amount of acrylamide cross linked by a methylene bridge ( $N,N'$  methylene Gel-Electrophoresis and Its Applications 17 bisacrylamide) allows formation of a cross linked gel with a highly-controlled porosity which is also mechanically strong and chemically inert. For separation of proteins, the ratio of acrylamide :  $N,N'$  methylene bisacrylamide is usually 40:1 while for DNA separation it is 19:1. Such gels are suitable for high-resolution separation of DNA and proteins across a large mass range.

#### 3.4.1 Preparation of polyacrylamide gel

- The listed protocol is for the preparation of a polyacrylamide with the dimensions of 15.5 cm wide by 24.4 cm long by 0.6 mm thick.
- Unpolymerized acrylamide is a neurotoxin and a suspected carcinogen; avoid inhalation and contact with skin. Always wear gloves when working with acrylamide powder or solutions.
- Methacryloxypropyltrimethoxysilane (bind silane) is toxic and should be used in a chemical fume hood.
- One glass plate will be treated with Gel Slick to prevent the gel from sticking and the shorter glass plate will be treated with bind silane to bind the gel. The two plates must be kept apart at all times to prevent cross-contamination.
- To remove the glass plate treatments (Gel Slick or bind silane), immerse the plates in 10% NaOH solution for one hour. Thoroughly rinse the plates with deionized water and clean with a detergent.
- The gel may be stored overnight on a paper towel saturated with deionized water and plastic wrap are placed around the well end of the gel to prevent the gel from drying out.



#### Staining

Protein is usually stained with the dye coomassie blue. Less sensitive protein dyes include ponceau red and amido black. Ponceau red has the advantage that it stains reversibly and may be removed from the protein to allow subsequent analysis (e.g. immunostaining).

### Staining

One of the most important aspects of gel electrophoresis technique is staining. Once sample molecules have separated in the gel matrix it is necessary to visualize their position. This is achieved by staining with an agent appropriate for the sample

Stain	Use	Detection limit (ng)
Amido black	Proteins	400
Coomassie blue	Proteins	200
Ponceau red	Proteins (reversible)	200
Bis-1-anilino-8-Naphthalene sulphonate	Proteins	150
Nile red	Proteins (reversible)	20
SYPRO orange	Proteins	10
Fluorescamine	Proteins (treated prior to electrophoresis)	1
Silver chloride	Proteins/DNA	1
SYPRO red	Proteins	0.5
Ethidium bromide (EtBr)	DNA/ RNA	10

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