LABROTORY INSTUMENTATION AND TECHNIQUES

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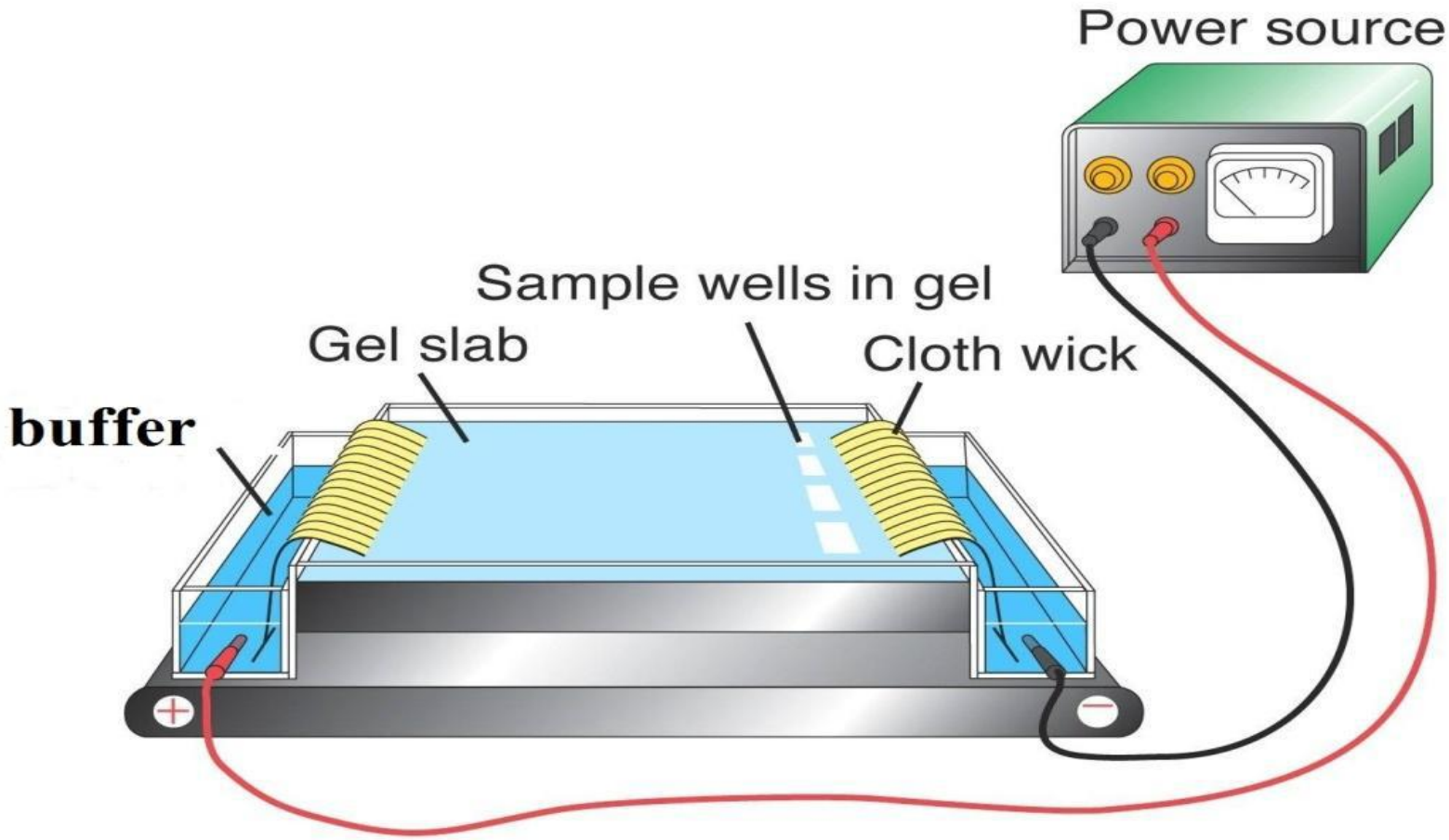
LECTURE NINE
ELECTROPHORESES

Electrophoresis: is a technique used to separate and sometimes purify charged macromolecules especially proteins and nucleic acids that differ in size, charge or shape by applying an electrical current.



The direction and speed of Electrophoresis depends upon several factors such as:-

a. Net charge of the molecule: charged molecules migrate and repel from electrode of a same charge and attract to electrode of opposite charge. DNA molecules always carry a negative charge. Proteins, on the other hand, carry a net charge that depends on the isoelectric point Ip (a specific pH at which the protein molecule will carry neutral net charge) and the pH of the buffer.

b. Size and shape of the molecule: small (low molecular weight) and circular molecules migrate faster than large and linear molecules, due to resistance and pores size of the matrix.

c. Electric field strength: increasing the voltage results in faster migration of the molecule.

d. Nature of the supporting matrix: the supporting matrix is a porus material with wells for loading the sample. The gel is immersed within a buffer The matrix can be composed of different materials such as cellulose acetate paper, polyacrylamide, agarose and starch gel each material with different pores size and consequently, different resolution.

f. The temperature of operation: electrophoresis at high voltages produces heat. Additionally, high-conductivity buffers generate more heat than low-conductivity buffers.

Types of electrophoresis & their techniques.

Electrophoresis can be broadly divided into 2 types as

1.Slab electrophoresis

2.Capillary electrophoresis.

The **slab method is the classical method which is widely used for industrial scale. It is slow, time consuming and bulky. Yet it is the sole method available for separation of proteins like enzymes, hormones, antibodies and nucleotides like DNA and RNA.This slab electrophoresis is further divided into 3 types based on the principle used for separation.**

a. Zone electrophoresis

b. Isoelectro-focusing

c. Immune-electrophoresis.

Zone electrophoresis: Here the charged particles are separated into different zones or bands.

This is of two types as

1.Paper electrophoresis.

2.Gel electrophoresis.

Paper electrophoresis is a techniques which employs a Whattman filter paper No.1 which is moistened by a buffer and then connected at two ends to two opposite charged electrodes. Then sample is applied on to one end and let for separation of components under electric field. After separation, the paper is dried and stained to get colored bands.

These colored bands are recognized for the nature of sample by comparing with the standard. For a sample of serum, 5 bands of proteins can be separated by paper electrophoresis.

Gel electrophoresis is a similar techniques wherein instead of paper, a gel made of agarose or SDS (sodium dodecyl sulphate).

The gel electrophoresis instrumentation

The separation is more efficient than paper type as the rate of movement is slow and area of separation is larger by thickness.

The sample is applied and subjected to electric field which can lead to separation of molecules. These molecules form bands and can be recognized by staining and comparing with standard sample bands. The method is more effective than paper and for instance from serum sample, 15 proteins bands can be isolated

Applications of electrophoresis:

1. To separate complex molecules: Many complex biological molecules like vitamins B12. antibiotics, proteins can be separated efficiently by electrophoresis. This is possible due to charge difference among the mixtures.

2. For analysis of nucleic acid molecules like RNA and DNA studies. These long chain molecules can be analyzed only after separation after electrophoresis.

This helps to determine the size or breaks in the DNA or RNA molecule.

This is due to differential rate of movement by molecules based on their weight. Those molecules with higher molecular weight move slower. While those with small weight move faster. Also the size of the molecule also influences the movement. The bigger size molecule experience more friction than smaller ones in motion. These molecules migrate at different speed and to different lengths based on their charge, mass and shape.