FAQ's

Q1. Define electrophoresis.

Ans: Electrophoresis is defined as the migration of charged particles or molecules in a medium under the influence of an applied electric field.

Q2. What are the main aims of carrying out electrophoresis?

Ans: The main aims of carrying out electrophoresis are: (i) to determine the number, amount and mobility of components in a given sample and/or to separate them, and (ii) to obtain information about the electrical double layers surrounding the particles.

Q.3. What is the basic principle of electrophoresis?

Ans: Electrophoretic separation is performed by injecting a small band of the sample into an aqueous buffer solution contained in a narrow tube, a flat porous supporting medium such as paper or a semi-solid gel. The high voltage is applied across the length of the buffer by means of a pair of electrodes located at its each end. This field causes ions of the sample to migrate towards the opposite charged electrodes. The rate of migration of a given species depends on its charge and its size. Separations are then based on differences in charge-to-size ratios for the various analytes in a sample. The larger this ratio, the faster an ion migrates in the electric field.

Q.4. Briefly, discuss the factors affecting electrophoretic mobility?

Ans: The factors that affect electrophoretic mobility are as under:

(1) **The sample: C**harge/mass ratio of the sample dictates its electro-

phoretic mobility. The mass consists of not only the size (molecular weight) but also the shape of the molecule.

(2) **The electric field:** The rate of migration under unit potential gradient is referred to as mobility of the ion. An increase in the potential gradient increases the rate of migration.

(3) **The medium:** The inert supporting medium used for electrophoresis can exert adsorption and/or molecular sieving effects on the particle thereby influencing its rate of migration.

(4) **The buffer:** Apart from maintaining the pH of the supporting medium, the buffer can affect the electrophoretic mobility of the sample in various ways.

i) **Composition.** The buffer can affect electrophoretic mobility if it is able to bind to component(s) of the sample being separated.

ii) **Ionic strength.** The increased ionic strength of the buffer results in slower migration of the sample components and a decrease in ionic strength, on the other hand, leads to a faster separation.

iii) **pH.** Increase in pH increases ionization of organic acids and a decrease in pH increases ionization of organic bases. For an ampholyte such as an amino acid, which has both acidic and basic properties, both the above effects apply.

Q.5. What is difference between free electrophoresis and zone electrophoresis?

Ans: The main difference between free and zone electrophoresis is that: In free electrophoresis no stabilizing media is used, while as, in *zone* electrophoresis stabilizing media is used.

Q.6. What are different types of free electrophoresis?

Ans: Free electrophoresis is divided into two main techniques:

(a) **Microelectrophoresis:** This electrophoretic technique involves the observation of motion of small particles in an electric field with a microscope (such as R. B. Cs, neutrophils, bacteria etc.). The suspension is contained in a closed system composed of a thin-walled section for optical observations and of suitable electrode compartments.

(b) **Moving boundary electrophoresis:** In this method, the electrophoresis is carried out in a solution, without a supporting media. The sample is dissolved, the buffer and molecules move to their respective counter charge electrodes.

Q.6. Briefly, discuss paper electrophoresis?

Ans: Paper electrophoresis is a commonly used electrophoretic method for analysis and resolution of small molecules. Two different methods are used routinely to apply samples to the electrophoretic paper. In the dry application procedure, a sample of solutes dissolved in distilled water, or a volatile buffer, is applied as a small spot or thin stripe on a penciled "origin line" on the paper. Appropriate standards of known compounds are applied at other locations on the origin line. After the solvent containing the samples has evaporated, the paper is dampened with the electrophoresis

buffer, either by uniform spraying or by dipping and blotting the ends of the paper so that wetting of the paper from both ends meets at the origin line simultaneously. In the wet application procedure, samples dissolved as concentrated solutions in distilled water are applied to paper predampened with electrophoresis buffer.

Q.7. What are the advantages of using cellulose acetate as a medium for electrophoresis?

Ans: Following are the advantages of using cellulose acetate as a medium of electrophoresis:

(i) Cellulose acetate is chemically pure; it does not contain lignins , hemicelluloses or nitrogen.

(ii) Cellulose strips are translucent and this makes them suitable for direct photoelectric scanning for separated bands of components,

(iii) Because of the very low content of glucose these strips are suitable for electrophoresis of polysaccharides; upon staining by schiff's reagent, background staining is negligible.

(iv) It is not hydrophilic and thus holds very little buffer. This property is conducive for a better resolution in a shorter time.

Q.8. What is gel electrophoresis?

Ans: Gel electrophoresis is a technique used to separate mixtures of DNA, RNA or proteins according to molecular size. The gels are porous and the size

of pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. The separation thus not only depends on the charge of the molecule but also on its size. Therefore, it can be said that resolution of a sample is sharper and better in a gel than in any other type of medium.

Q.9. Define the term molecular sieve?

Ans: A molecular sieve is a material with pores (very small pores) of uniform size. These pore diameters are of the dimensions of small molecules, thus large molecules cannot be absorbed while small molecules can. Some examples of materials used as molecular sieve are activated charcoal, silica gel etc.

Q.10. Name the different type of gels and solubilizers used in electrophoresis?

Ans: The different type of gels and solubilizers used in electrophoresis are:

(a) **Gels:** Starch gel, agar, polyacrylamide, agrose-acrylamide, pectin etc.

(b) **Solubilizers:** Urea, sodium dodecyle sulphate, β-mercaptoethanol etc.

Q.11. What is Isoelectric focusing?

Ans: Isoelectric focusing is an electrophoretic technique that separates macromolecules on the basis of their isoelectric points (pI, pH

values at which they carry no net charge). As with SDS-PAGE, this process can be carried out in a "slab" format. A pH gradient is established in the polyacrylamide gel with the aid of ampholytes, which are small (~5000 Da) polymers containing random distributions of weakly acidic and weakly basic functional groups (e.g., carboxyl, imidazoles, amines, etc.).

Q.12. How is agrose gel prepared?

Ans: Agarose gel is prepared by dissolving the white agarose powder in an aqueous buffer containing EDTA and either Tris-acetate or Tris-borate as the buffering species (TAE or TBE buffer, respectively). When the sample is heated to just below boiling point, the agarose powder dissolves in the buffer to form a clear solution. As the solution slowly cools to room temperature, hydrogen bonding within and between the polygalactose units in the solution causes the formation of a rigid gel with a relatively uniform pore size.

Q.13. Name the two types of tracking dyes commonly used in agarose gel electrophoresis. What is their significance?

Ans: The two types of tracking dyes commonly used for agarose gel electrophoresis are, Bromophenol blue and xylene cyanole. These are used to monitor the progress of the electrophoresis. *Bromophenol* blue dye will migrate at the same rate as a DNA molecule of about 500 base pairs, while *xylene cyanole* dye will migrate at the same rate as a DNA molecule of about 4000 base pairs.

Q.14. How are nucleic acids visualized in agarose gel electrophoresis?

Ans: In order to visualize the nucleic acids following the electrophoretic separation, *ethidium bromide*, a fluorescent dye that has the ability to intercalate between the stacked bases of nucleic acid duplexes (i.e., the double helix of DNA) is used. After electrophoresis, the gel is placed in a solution of TBE or TAE buffer containing ethidium bromide, which diffuses into the gel and associates with the nucleic acids. Following a short destaining period in buffer without ethidium, the gel is briefly exposed to ultraviolet (UV) light (256–300 nm), revealing the nucleic acid fragments as orange or pink fluorescent bands in the gel.

Q.15. Write few applications of electrophoresis?

Ans: Following are the applications of electrophoresis:

(i) Electrophoresis plays a vital role in the separation of nucleic acids and proteins in the field of genomics and proteomics.

(ii) Electrophoresis such as capillary electrophoresis can be used to analyze inorganic ions (Na, K, Ca and Mg) in foods like, pretzels, breadcrumbs, peanut butter, parsley and Parmesan cheese.

(iii) It can also be used to analyze drinking water for the presence of nitrate and nitrite and chloride, sulfate, nitrate and fluoride.

(iv) Electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The assay can be used for qualitative as well as quantitative analysis.