VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED 'A' GRADE

Topic:Basic principles of electrophoresis,IEF,SDS-PAGE and Chromatography

Course Title:Enzyme

Paper:CC4

Unit:III

Semester:2

Name of the Teacher:Nineesha Sen Banerjee

Name of the Department:Biochemistry

Electrophoresis

Electrophoresis is the migration of ions in an electric field. $F_{electric}$, on an ion of charge q in an electric field of strength E,

$$F_{electric} = qE$$

The resulting electrophoretic migration of ions through the solution is opposed by a frictional force

$$F_{friction} = fv ,$$

where, v is the rate of migration of ions, f is the frictional coefficient.

In a constant electric field, the forces on the ion balance each other.

$$\therefore qE = vf$$

$$\therefore \frac{q}{f} = \frac{v}{E} = u = \text{electrophoretic mobility}$$

polypeptide chain.

In polyacrylamide gel electrophoresis (PAGE), are made by free radical induced polymerisation (APS and TEMED) of acrylamide and N, N'-methylene bis acrylamide in the buffer of choice.

$$[e^-] > [\text{protein} - \text{SDS}] > [\text{Glycinate}]$$

17/4/19

$\frac{10x}{M.W.} \cdots \frac{A}{\varepsilon_{molar}} = \frac{10x}{M.W.}$ $\quad A = \varepsilon c l$ ← 1cm

$= \varepsilon c$

$x = \frac{A \times M.W.}{\varepsilon_{molar} \times 10}$ $\quad \frac{A}{\varepsilon_{molar}} = [M]$;

$\frac{A}{\varepsilon_{1\%}} = [\%] = x$

$x = \frac{A \times M.W.}{\varepsilon_{molar} \times 10} = \frac{A}{\varepsilon_{1\%}}$

$\therefore \varepsilon_{molar} \times 10 = \varepsilon_{1\%} \times M.W.$

Liquid chromatography

To minimise loss of biological activity, separations are often carried out in aqueous buffers below room temperature. Low temperatures are especially important in chromatography of cell extracts, for example, protein purification. This reduces protease activity which might ~~otherwise~~ otherwise destroy the protein of interest. Chromatography with liquid mobile phase is called liquid chromatography.

Example: In partition chromatography, the sample separates into individual components as it passes through the stationary phase. In ion ~~exchange~~ exchange chromatography, the sample components are eluted by means of a gradient of competing salt counter ions after all the sample has been loaded.

Gas chromatography

When biomolecules are sensitive to high temperatures that can ~~be~~ lead to destruction of structure and function. However, some molecules of importance may be converted to ~~their~~ derivatives that are structurally stable though volatile in the temperature range 200–250°C.

Example: Methylated esters of fatty acids.

A second category of molecules, example ethanol is stable and ~~so~~ volatile at a lower temperature without derivatisation.

Both of these groups of molecules may be analysed by gas chromatography, also called gas-liquid chromatography This is a form of partition chromatography in which a volatile sample is carried in an inert gas mobile phase ~~such~~ (carrier gas) such as nitrogen, helium or argon, and ~~applied~~ applied to a narrow ~~colum~~ column ranging in length from 1-30 metres that contains liquid stationary phase. Sample when injected in the system, the injection ~~po~~ port is held approximately 10° above the column temperature to ensure efficient ~~volatilis~~ volatilisation.