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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT 12. Electrophoresis

Content outline

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Elecctrophoresis: Definition

The term electrophoresis describes the migration of a charged particle under the influence of an electric field. Many important biological molecules, such as amino acids, peptides, proteins, nucleotides and nucleic acids, possess ionisable groups and therefore will migrate either to the cathode or to the anode, depending on the nature of their net charge.

How charged species are separated?

When a potential difference (voltage) is applied across the electrodes, it generates a potential gradient, E, which is the applied voltage, V, divided by the distance, d, between the electrodes. When this potential gradient E is applied, the force on a molecule bearing a charge of q coulombs is Eq newtons. It is this force that drives a charged molecule towards an electrode. However, there is also a frictional resistance that retards the movement of this charged molecule. The velocity, n, of a charged molecule in an electric field is therefore given by the equation:

$$\nu = \frac{Eq}{f}$$

Electrophoretic mobility (μ) = It is the ratio of the velocity of the ion to field strength (*V/E).*

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.

Frictional force is dependent upon following factors:

a. hydrodynamic size of the molecule,
b. the shape of the molecule,
c. the pore size of the medium in which electrophoresis is taking place
d. the viscosity of the buffer

Major problem faced during electrophoresis

Generation of heat during electrophoresis is a major problem. This heat generation is due to generation of current due to migration of buffer ions and sample ions. Ohm's law expresses the relationship between current (I), voltage (V) and resistance (R).

$$\frac{V}{I} = R$$

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

$$W = I^2R$$

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 is rather sensitive to heat. This may include denaturation of proteins (and thus the loss of enzyme activity).
- A decrease of buffer viscosity, and hence a reduction in the resistance of the medium.

Test your Understanding

Which technique separates charged particles using electric field?

a. Hydrolysis
b. Electrophoresis
c. Protein synthesis
d. Protein denaturing

Electrophoresis was developed by:

a. Tswett
b. Tiselius
c. Sanger
d. Tiselius and Twsett working Together

Electrophoretic mobility (μ) is

a. It is the ratio of the velocity of the ion to field strength (V/E)
b. It is the velocity of ion in the gel
c. Magnitude of charge and mass of molecule
d. Magnitude of charge shape and mass of molecule

The speed of migration of ions in electric field depends upon:

a. Magnitude of charge and shape of molecule
b. Magnitude of charge shape and mass of molecule
c. Magnitude of charge and mass of molecule
d. None of the above

The heating of electrophoretic medium is due to

a. Electric current
b. Magnetic current
c. Water current
d. Under current

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.