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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OFENGINEERING & TECHNOLOGY

LT 16 Paper electrophoresis

Content Outline

1. Paper electrophoresis
2. Pulse Field Gel Electrophoresis
3. Test your understanding
4. Reference & Further reading

Paper Electrophoresis

•Paper electrophoresis (PE) is useful for the separation of small-charged molecules, such as amino acids and small proteins using a strip of paper (chromatography paper).

•In this technique, the motion of colloidal particle of solution occurs leading to subsequent separation along the paper strip (Figure 9). PE is easier in comparison to gel electrophoresis. It does not require matrix preparation and it does not contain charges that interfere with the separation of compounds.

•A strip of filter paper is moistened with buffer and the ends of the strip are immersed into buffer reservoirs containing the electrodes. The samples are spotted in the center of the paper and high voltage is applied. Application of high voltage causes less diffusion of small molecules giving better resolution and it take less time to complete the process. Spots migrate according to their charges. After electrophoresis, separated components can be detected by variety of staining techniques, depending upon their chemical composition.

Paper Electrophoresis in Figure

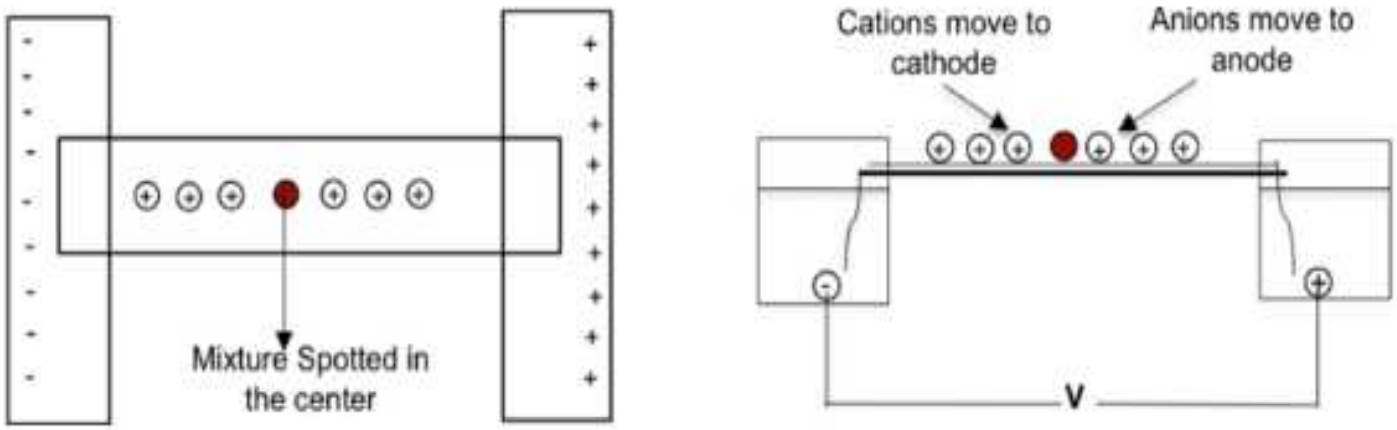


Figure 9. Schematic representation of Paper Electrophoresis

Paper Electrophoresis in Figure

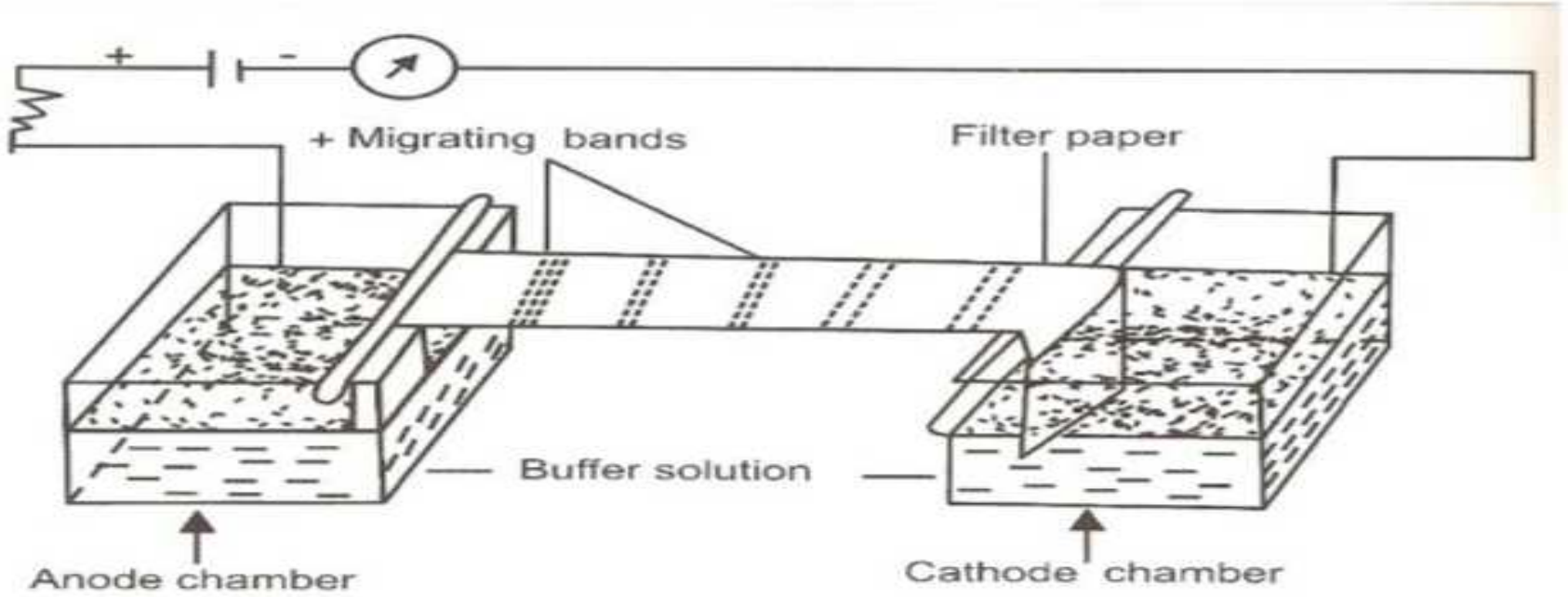


Fig. 28.1: Paper electrophoresis (one-dimensional)

Applications

Applications

•Clinical applications of PE include study of sickle cell disease, hemoglobin C abnormalities, and separation of blood clotting factors and serum plasma proteins from blood sample. It has also been used in separation and identification of alkaloids.

•PE can also be used for testing suitability of municipal water supplies, toxicity of water, and other environmental components.

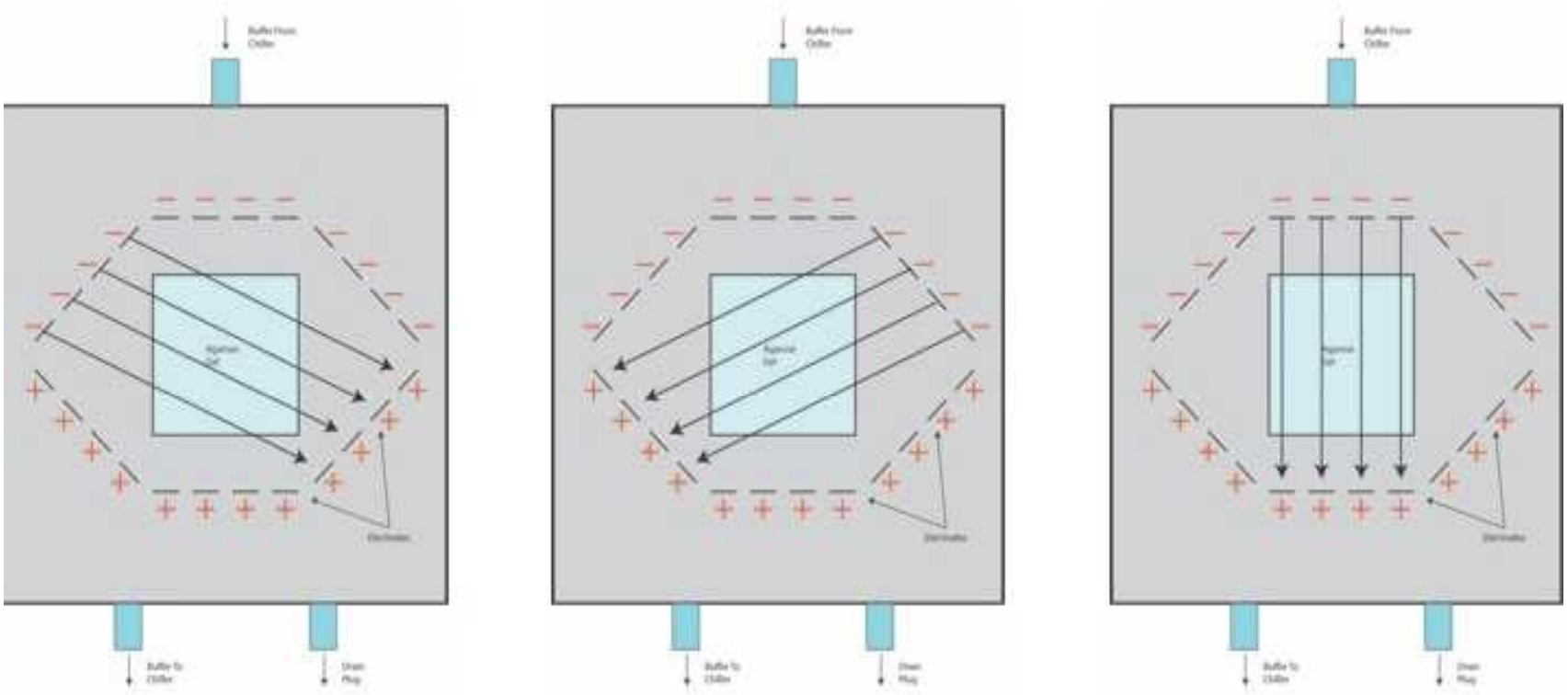
•Drug-testing industry uses paper electrophoresis to determine presence of illegal or recreational drugs in job applicants and crime suspects. PE has been used since 1950s by the investigators and in forensics to analyze inks used in currency to check the counterfeiters

Pulse field gel electrophoresis

•*Pulsed* field *gel electrophoresis* is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a *gel* matrix an electric field that periodically changes direction. It can be used to separate entire genomic DNA or DNA fragments up to length of 2×10^3 kb. PFGE is used as sequencing gel; therefore Sequencing gels are run in the presence of denaturing agents, urea and formamide. Since it is necessary to separate DNA molecules that are very similar in size, DNA sequencing gels tend to be very long (100 cm) to maximise the separation achieved.

•The method basically involves electrophoresis in agarose where two electric fields are applied alternately at different angles for defined time periods (e.g. 60 s). Activation of the first electric field causes the coiled molecules to be stretched in the horizontal plane and start to move through the gel. Interruption of this field and application of the second field force the molecule to move in the new direction. In this way, with continual reversing of the field, smaller molecules draw ahead of larger molecules and separate according to size.

Pulse field gel electrophoresis : Operation



1st Pulse 2nd Pulse 3rd Pulse

An example of a single PFGE cycle, the arrows indicate which electrodes are active during a certain pulse cycle. The DNA is pulled at different angles throughout the program, with the net result being the DNA moving slowly towards the bottom of the gel.

Application

•Sequencing of genomic DNA

•PFGE has proved particularly useful in identifying the course of outbreaks of bacterial foodborne illness (e.g. Salmonella infections).

Test your understanding

Support medium used in paper electrophoresis is

a. Butter paper

b. Newspaper

c. Velvet paper

d. Chromatographic paper

Choose the correct statement regarding paper electrophoresis

a. Chromatographic paper is used as support medium

b. useful for the separation of small-charged molecules, such as amino acids and small proteins

c. Both (a) and (b)

d. Neither (a) nor (b)

Pulse Field gel electrophoresis (PFGE) can be used to separate

a. DNA fragments of 2×10^3 kb length

b. DNA fragments of 1-10 kb length

c. DNA fragments of 10-50 kb length

d. None of these

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.