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DEPARTMENT OF BIOTECCHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT 13: Support media & Boundary Electrophoresis

Content outline

1. Support media
    a. Agarose
    b. Polyacrylamide
2. Boundary Electrophoresis

Support media

The purpose of using support media foe electrophoresis is to cut down convection currents and diffusion so that the separated components remain as sharp zones. Initially, starch gels were used for nowdays agarose gel or polyacrylamide gels are used for electrophoretic techniques.

Agarose gel

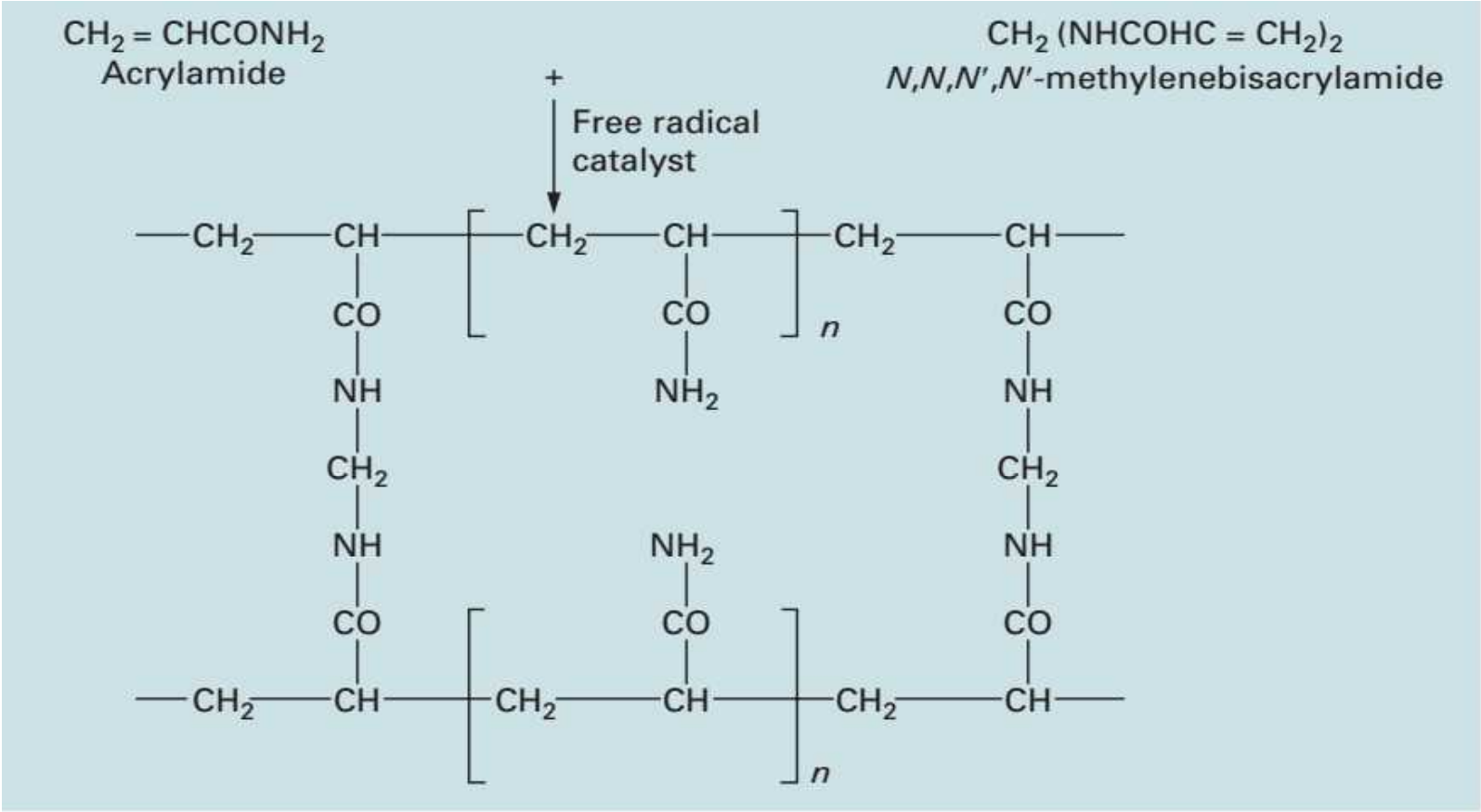
Agarose is a linear polysaccharide (average relative molecular mass about 12 000) made up of the basic repeat unit agarobiose, which comprises alternating units of galactose and 3,6-anhydrogalactose. Agarose is usually used at concentrations of between 1% and 3%. Agarose gels are formed by suspending dry agarose in aqueous buffer, then boiling the mixture until a clear solution forms. This is poured and allowed to cool to room temperature to form a rigid gel. The gel formation is due to formation of inter- and intramolecular hydrogen bonding within and between the long agarose chains The pore size in the gel is controlled by the initial concentration of agarose; large pore sizes are formed from low concentrations and smaller pore sizes are formed from the higher concentrations.

Agarose gels are used for the electrophoresis of both proteins and nucleic acids. For proteins, the pore sizes of a 1% agarose gel are large relative to the sizes of proteins. Such large pore gels are also used to separate much larger molecules such as DNA or RNA, because the pore sizes in the gel are still large enough for DNA or RNA molecules to pass through the gel.

There are two types of gel electrophoresis methods: (i) Vertical slab gel (ii) Horizontal slab gel Horizontal slab gels are invariably used for isoelectric focussing or immunoelectrophoresis in agarose. Horizontal gels are also used routinely for DNA and RNA gels, although vertical systems have been used by some workers.

Polyacrylamide gels

Electrophoresis in acrylamide gels is frequently referred to as PAGE, being an abbreviation for polyacrylamide gel electrophoresis. Cross-linked polyacrylamide gels are formed from the polymerisation of acrylamide monomer in the presence of smaller amounts of N,N0-methylene-bisacrylamide (normally referred to as ‘bis’-acrylamide). The polymerisation of acrylamide is an example of free-radical catalysis, and is initiated by the addition of ammonium persulphate and the base N,N,N0,N0-tetramethylenediamine (TEMED). TEMED catalyses the decomposition of the persulphate ion to give a free radical (i.e. a molecule with an unpaired electron).

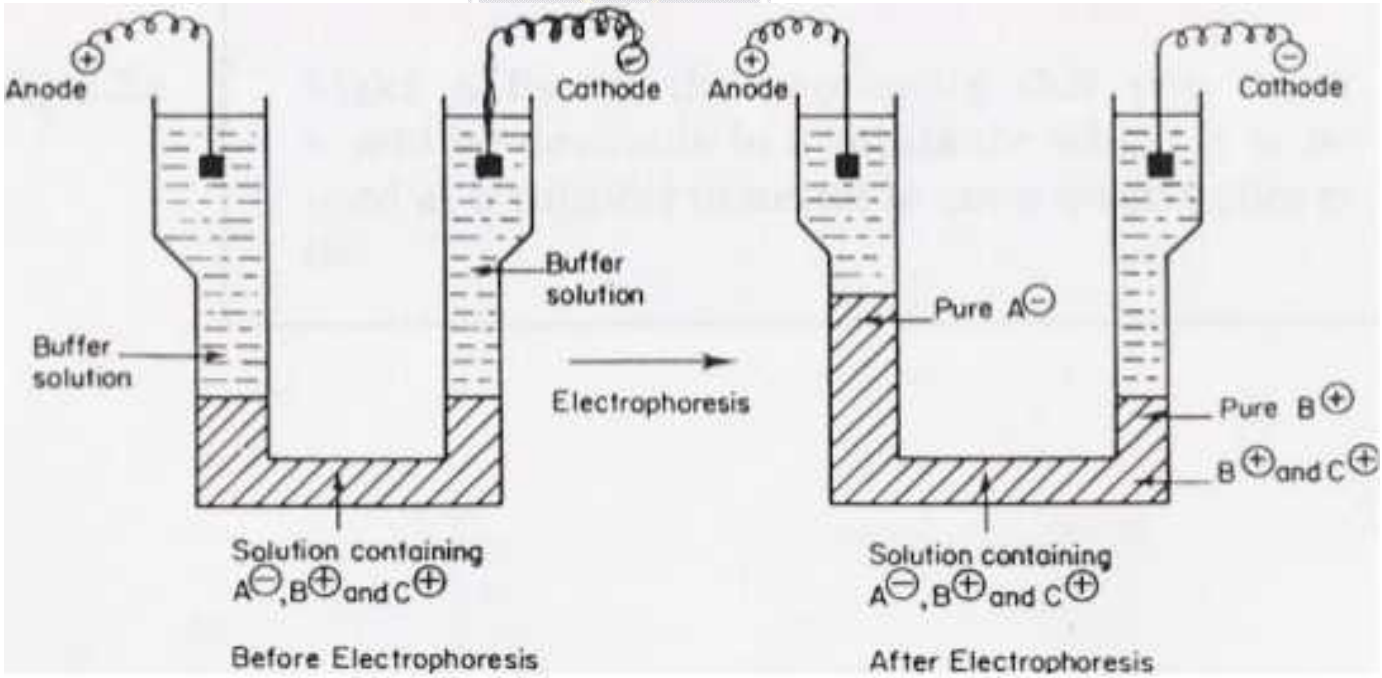


The formation of a polyacrylamide gel from acrylamide and bis-acrylamide

Boundary electrophoresis

➢This method of electrophoresis allows charged species to migrate in a free moving solution in the absence of a supporting medium. It was first performed by Arne Tiselius.

Samples are fractionated in a U-shaped tube that has been filled with unstabilized buffer. An electric field is applied by means of electrode at the end of the U-tube. The charge species began to migrate according to their mobilites through the solution. Detection of separated band is done by measuring the refractive index of solution using refractometer.



➢Capillary electrophoresis, Isoelectric focusing and Immunoelectrophoresis are example of moving boundary electrophoresis.

➢**Disadvantages of Moving Boundary electrophoresis-**The resolution of the technique is very low due to the mixing of the sample as well as over-lapping of the sample components. The electrophoresis technique is not good to separate and analyze the complex biological sample instead it can be used to study the behavior of the molecule in an electric field

Test your Understanding

Which is true in case of Boundary electrophoresis?

a. It involves migration of charge in a free moving solution
b. It involve migration of charge sample in gel medium
c. Neither (a) nor (b)
d. All of the above

Select the true statement regarding boundary electrophoresis

a. It achieves good resolution
b. The resolution capacity is low
c. Mixing and overlapping of samples are common phenomenon
d. Both (b) and (c)

Support media used during electrophoresis is/are

Agarose

Polyacrylamide

Cellulose Paper

All of the above

How do you control the pore size of agrose/ polyacrylamide gel?

a. By varying the concentration of gel powder
b. By varying the electric current
c. By varying the magnetic field

References & Further reading

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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.