



10.3 MATRICES USED IN GEL ELECTROPHORESIS

The type of matrix used for preparing a gel in the gel electrophoresis must have two basic features, it should be inert towards the biomolecule and it must be capable of forming a uniform pore-containing matrix. Two commonly known gel matrices are used in Biology, Agarose which is of biological origin (isolated from red algae) and polyacrylamide of chemical origin (chemically synthesized). Although the pore size of both type of gels may be modulated by changing different matrix concentrations, The handling becomes difficulty under very low or very high concentration, which limits the usage of these matrices at any preferred concentration. The agarose gels contain large pore size while polyacrylamide gels produce small pore size therefore for the separation of molecules with larger difference in molecular weight, Agarose is preferred while for smaller difference in molecular weight polyacrylamide is preferred.

Now, let us discuss in detail about the chemical nature and polymerization of two matrices

Agarose – highly purified polysaccharide derived from agar (extracted from seaweed), long sugar polymers held together by hydrogen and hydrophobic bonds.

Acrylamide ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$) Polyacrylamide gel structure held together by covalent cross-links

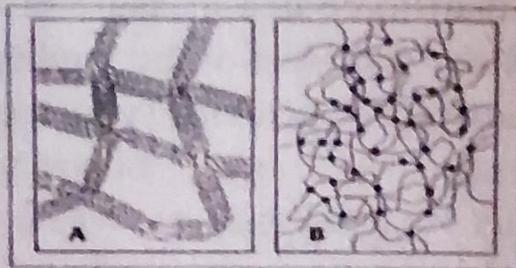
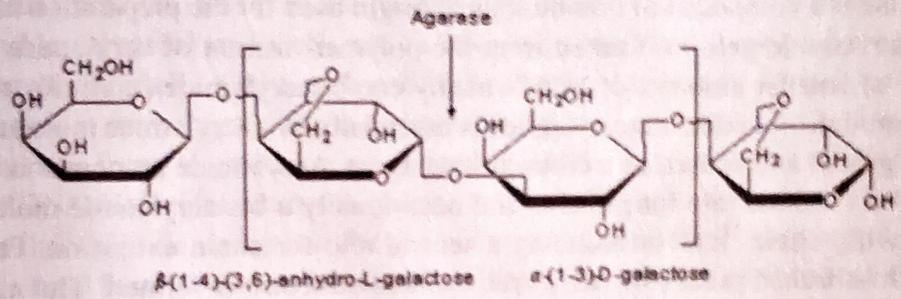


Fig. 10.2. Difference in the porosity of agarose and acrylamide gels a. agarose gels forms by non-covalent hydrogen bonds between long sugar polymers. B. acrylamide gels have covalent cross links between polymers.

Agarose

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 one of the components of agar that is a mixture of polysaccharides isolated from red algae (*Gelidium* and *Gymnodinium* sp). The polymerization of agarose gel is an spontaneous process, and mere suspending dry agarose in aqueous buffer, then boiling the mixture until a clear solution and cooling down to room temperature to form a rigid gel. The gelling properties are attributed to both inter- and intramolecular hydrogen bonding within and between the long agarose chains. This cross-linked structure gives the gel good anti-conventional properties. 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. Although essentially free from charge, substitution of the alternating sugar residues with carboxyl, methoxyl, pyruvate and especially sulphate groups occurs to varying degrees. This substitution can result in electro-endosmosis during electrophoresis and ionic interactions between the gel and sample in all uses, both unwanted effects. Agarose is therefore sold in different purity grades, based on the sulphate concentration, the lower the sulphate content, the higher and the purity.

Agarase Specificity



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. Agarose gels are therefore used in techniques such as flat-bed isoelectric focusing.

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. Now, however, the pore size and molecule size are more comparable and fractional effects begin to play a role in the separation of these molecules.

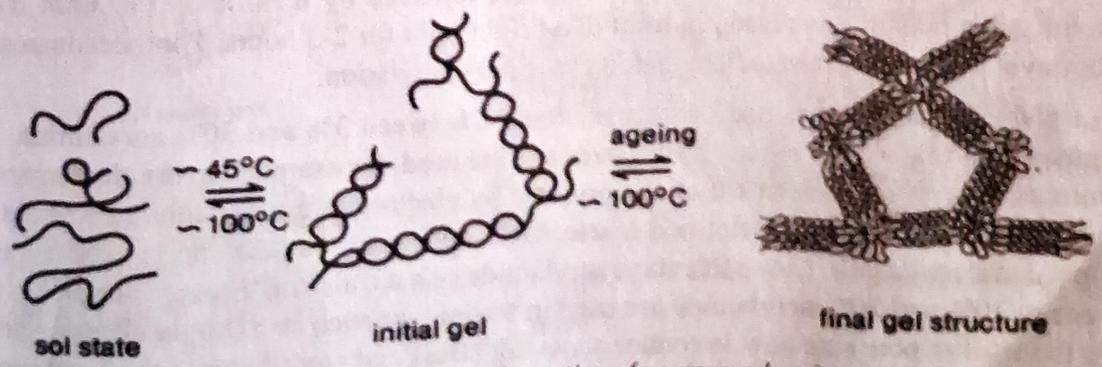


Fig. 10.3. Topological changes in the formation of agarose gels, microscopic view of gel.

Acrylamide

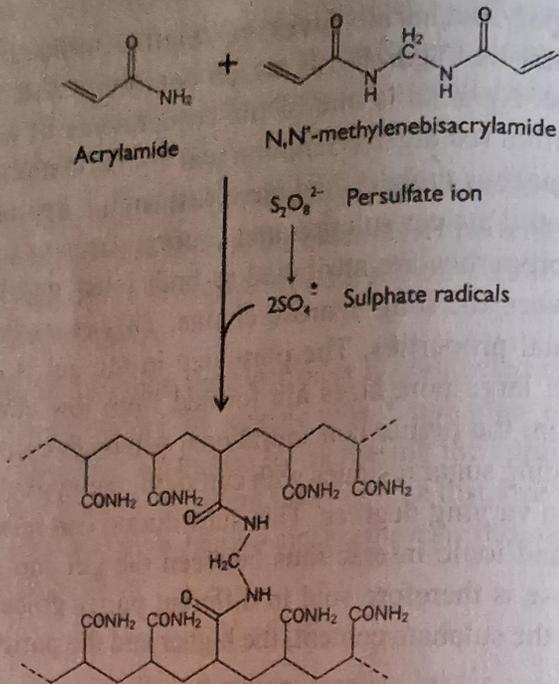


Fig. 10.4. Chemical reaction involving polymerization of acrylamide gel.

Acrylamide is a compound of non-biological origin used for the preparation of gels. linked polyacrylamide gels are formed from the polymerization of acrylamide monomers in the presence of smaller amounts of N, N' -methylene-bisacrylamide (normally referred to as 'bis'-acrylamide). Note that bisacrylamide is essentially two acrylamide molecules linked by a methylene group, and is used as a cross-linking agent. Acrylamide monomer is polymerized in a head-to-tail fashion into long chains and occasionally a bis-acrylamide molecule is added into the growing chain, thus introducing a second site for chain extension. Proceeding in this way a cross-linked matrix of fairly well-defined structure is formed. The mechanism of polymerization of acrylamide is an example of free-radical catalysis, and is initiated by the addition of ammonium persulphate (APS) and the base N, N, N', N' -tetramethylethylenediamine (TEMED). TEMED catalyses the decomposition of the persulphate ion to give a free radical.

The polymerization of acrylamide is an exothermic reaction (i.e. heat is liberated) and a warning up of the gel solution as it sets can liberate air bubbles that become trapped in the polymerized gel.

Photopolymerisation is an alternative method that can be used to polymerise acrylamide gels. The ammonium persulphate and TEMED are replaced by Riboflavin (Vitamin B₂). When the gel is poured it is placed in front of a bright light for 2-3 hours. Photodecomposition of riboflavin generates a free radical that initiates polymerization.

Acrylamide gels can be made with a content of between 3% and 30% acrylamide. Low percentage gels (e.g. 4%) have large pore sizes and are used, for example, in the electrophoresis of proteins, where free movement of the proteins by electrophoresis is required without a noticeable frictional effect (in flat-bed isoelectric focusing or the stacking gel system of SDS-polyacrylamide gel). Low percentage acrylamide gels are also used to separate DNA. High percentage gels of between 10% and 20% acrylamide are used in techniques such as SDS-gel electrophoresis where the smaller pore size now introduces a sieving effect that contributes to the separation of proteins according to their size.