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Appendix B: Agarose Physical Chemistry

Agarose is a polysaccharide consisting of 1,3-linked β -D-galactopyranose and 1,4-linked 3,6-anhydro- α -L-galactopyranose (Figure 1). This basic agarobiose repeat unit forms long chains with an average molecular mass of 120,000 daltons, representing about 400 agarobiose units (1). There are also charged groups present on the polysaccharide, most notably pyruvate and sulfate. These residues are responsible for many agarose properties, and by careful selection of raw materials, these properties can be controlled to meet specific needs.

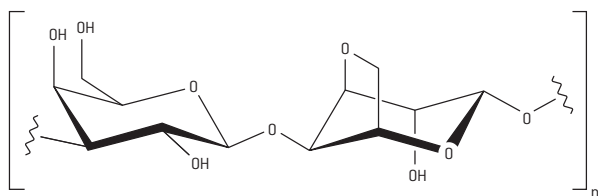


Figure 1: Agarobiose – Basic repeating unit of agarose.

Electroendosmosis (EEO) is a functional measure of the number of these sulfate and pyruvate residues present on the agarose polysaccharide (Figure 2). Electroendosmosis (2,3) is a phenomenon that occurs during electrophoresis when the anticonvective medium (the agarose in this case) has a fixed negative charge. In an electric field, the hydrated positive ions associated with the fixed anionic groups in the agarose gel migrate toward the cathode. Water is thus pulled along with the positive ions, and migration of negative molecules such as DNA is retarded (Figure 3). Electroendosmosis is quantitated by subjecting a mixture of dextran and albumin to electrophoresis, then visualizing them and measuring their respective distances from the origin. The amount of EEO (-mr) is calculated by dividing the migration distance of the neutral dextran (OD) by the sum of the migration distances of the dextran and the albumin (OD + OA): $-mr = OD / (OD + OA)$.

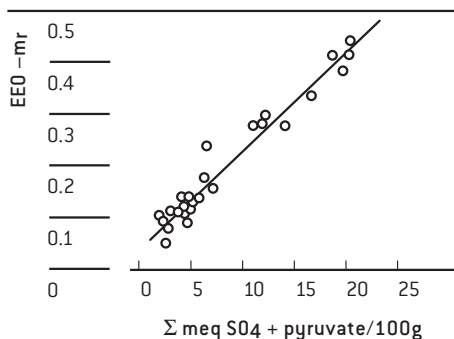


Figure 2: Dependence of electroendosmosis on total anionic residues in agarose.

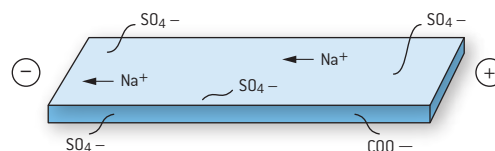


Figure 3: Illustration of EEO. Anionic groups are fixed to the matrix and cannot move. Corresponding cations can move and sweep toward the cathode with their associated water of hydration.

The mechanism for gelation of agarose was first suggested by Rees (4) and later demonstrated by Arnott, et al. (5). It involves a shift from a random coil in solution to a double helix in the initial stages of gelation, and then to bundles of double helices in the final stage (Figure 4). The average pore size varies with concentration and type of agarose, but is typically 100 to 300 nm (6).

Gelation Mechanism

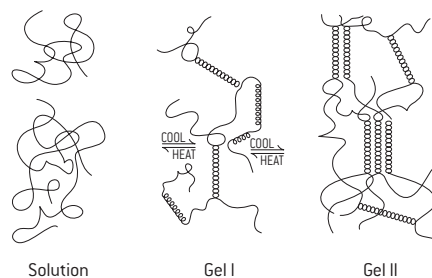


Figure 4: Gelation of agarose by formation of double helices connected in three dimensions by zones of random coil configuration.

One of the most important factors contributing to the success of agarose as an anticonvection medium is its ability to exhibit high gel strength at low concentrations ($\leq 6\%$). Gel strength is defined as the force, expressed in g/cm^2 , that must be applied to fracture an agarose gel of a standard concentration. As there are several test methods used to measure gel strength, a direct comparison of gel strength values between different manufacturers is sometimes difficult.

The gel strength of a specific lot of agarose will decrease over time because of the spontaneous hydrolysis of the agarose polysaccharide chains. This loss of gel strength can be particularly noticeable after 5 years from the manufacturing date.

Agents that disrupt hydrogen bond formation (chaotropic agents such as urea and potassium iodide) will decrease the melting temperature, gelling temperature and gel strength of agarose gels, or even inhibit the formation of the gel. Since some electrophoretic procedures call for the incorporation of urea into the gel, it is important to be aware of this effect.

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Continued

The energy needed to melt an agarose gel increases as the gel concentration increases. The gelling temperature of an agarose gel is also influenced by the gel concentration [Figure 5]. For this reason, gelling or remelting temperatures are expressed at a given agarose concentration. This property is of practical value since it is possible to vary gelling and melting parameters by using lower or higher concentrations of agarose. The dependence of gelling and melting temperatures on concentration is most pronounced at concentrations less than 1%.

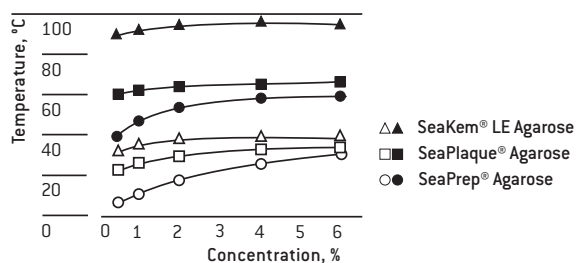


Figure 5: Effect of concentration on gelling (open symbols) and melting (solid symbols) temperatures of agaroses.

The agarose polysaccharide also contains uncharged methyl groups. The extent of natural methylation is directly proportional to the gelling temperature. Unexpectedly, synthetically methylated agaroses have lower, rather than higher, gelling temperatures (Figure 6, lower curve), and the degree of synthetic methylation is inversely proportional to the melting temperature. A subsequent study demonstrated that a number of simple derivatives have the same effect as synthetic methylation on gelling temperature [Figure 7]. Because of manufacturing concerns, hydroxyethylation is preferred for large scale derivatization of agarose.

The major use of low gelling temperature agarose, such as SeaPlaque® GTG® and NuSieve® GTG® Agarose, is to take advantage of the low melting point rather than the low

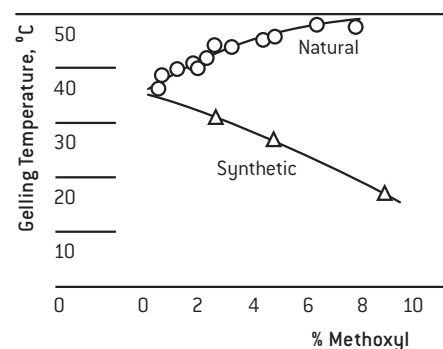


Figure 6: Dependence of agarose gelling temperature on natural and synthetic methylation.

gelling temperature. Because these agarose types have a melting temperature of $\leq 65^\circ\text{C}$, it is possible to remelt the agarose without melting the DNA double helix. Thus, slices of SeaPlaque® GTG® or NuSieve® GTG® Agarose gels containing DNA can be melted and the nucleic acids manipulated directly in the remelted agarose.

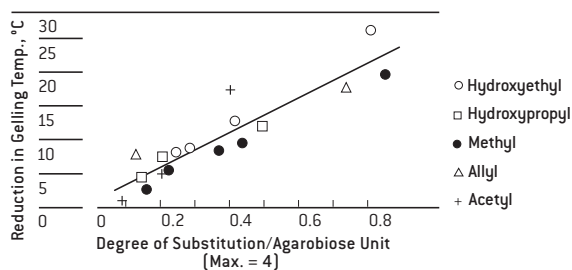


Figure 7: Effect of various substituents on agarose gelling temperature.

References

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