CROSS-LINKED LINEAR POLYSACCHARIDE POLYMERS AS GELS FOR ELECTROPHORESIS

This is a division of application Ser. No. 07/998,299, filed Dec. 30, 1992, now U.S. Pat. No. 5,371,208.

FIELD OF THE INVENTION

This invention relates to novel gels and the use of these gels as a matrix for electrophoretic separation of molecules.

BACKGROUND OF THE INVENTION

Electrophoresis is a process for separation of charged species based on different mobilities of these species in electric field. The mobilities depend on electrophoresis medium, electric field strength and characteristics of ions themselves, including net surface charge, size and shape. Small species, like metal ions, as well as large species such as viruses have been separated by electrophoretic techniques. However, electrophoresis is currently used mostly for separation of biological macromolecules, including proteins, nucleic acids and their derivatives. The process is usually carried out by forcing the molecules to migrate through an aqueous gel as the electrophoresis medium. The gels may be composed of natural or synthetic polymers. Agarose is the most widely used natural material and polyacrylamide gels represent the most common synthetic matrix. The gels are run essentially in two types of electrophoretic units, including vertical and horizontal ones. In horizontal units the contact between the electrodes and the gel may be established directly or by means of wicks. Alternatively, the gel may be immersed in a buffer which serves as a conductive medium between electrodes and the gel. This format is known as submerged gel electrophoresis and it is the simplest to operate. Submerged gel electrophoresis is widely used for analysis of nucleic acids and agarose gels are the mostly used matrix.

A new synthetic matrix has been introduced for analysis of proteins and nucleic acids by Kozulic et al (U.S. patent application Ser. No. 328,123, Analytical Biochemistry 163 (1987) 506–512 and Analytical Biochemistry 170 (1988) 478–484). The matrix is based on an acrylic monomer, N-acryloyl-tris(hydroxymethyl)aminomethane (NAT). The poly(NAT) gels were found to be more porous than polyacrylamide gels but less porous than agarose gels. The gels were particularly suitable for separation of DNA molecules in size range from about **50** to a few thousand base pairs. However, as described in Kozulic (PCT/EP 92/00368), which is incorporated herein by reference, it was noticed that resolution of DNA in the poly(NAT) gels run in the submerged gel electrophoresis mode was never as good as in the vertical format. Subsequently, it was surprisingly observed that separated DNA bands in the submerged gel were bent, that is declined from the vertical axis. Such bending is detrimental for resolution because on a gel record, made by a camera positioned above the gel, the separated bands appear broad and diffuse. The cause of this bending was related mostly to ionic compositions of the gel and electrophoresis buffer. The bending could be eliminated or greatly reduced by adjustment of the ionic composition of the gel, as disclosed in the above cited document. The adjustment needed to be done each time after a considerable change in total monomer concentration, electrophoresis buffer or gel dimensions. Since these three variables are often changed in a research laboratory to improve resolution over a certain

size range, it is apparent that a great amount of work would be saved if there were gels giving good DNA resolution in the range of up to a few thousand base pairs without being hampered by bending of bands at different gel concentrations and dimensions.

Agarose gels comprising about 0.6 to 1 percent polymer are suitable for separation of DNA molecules in size range from a few thousand to a few tens of thousand base pairs. The size range can be extended to several million base pairs by pulsed field electrophoresis (Cantor et al. Ann. Rev. Biophys. Biophys. Chem. 17 (1988) 287–304). Smaller DNA molecules require higher agarose concentrations for good resolution, as generally known in prior art. However, more concentrated agarose gels are difficult to prepare due to a high viscosity of agarose solutions. Furthermore, visualization of separated bands is difficult due to gel opacity. Derivatization of hydroxyl groups of agarose, as disclosed in U.S. Pat. No. 3,956,273 to Guisely, reduces viscosity of agarose solutions as well as gel opacity. Such hydroxyethylated agarose derivatives are commercially available products known under the trade name SeaPlaque and NuSieve (FMC Corporation). NuSieve agarose is typically used at polymer concentrations from about 2 to 8% and improved resolution of small DNA in this agarose has been reported (Dumais and Nochumson, BioTechniques, 5 (1987) 62). However, separated DNA bands were bent, although to a lesser degree than in poly(NAT) gels, also in NuSieve agarose gels containing as little as 4% of polymer (Kozulic, PCT/EP 92/00368). The bending could be reduced by adjustment of ionic composition of the gel, but as noted above it would be preferable to have a gel which does not require the adjustment.

The bending effect described was influenced by several factors but it was related mostly to difference in conductivity between the electrophoresis buffer and the gel immersed in that buffer. This difference in conductivity is caused by resistance of gel polymers to migration of buffer ions. The resistance, and therefore the difference in conductivity, may be presumably reduced by lowering the polymer concentration of the gel. However, there are many reports in prior art showing that lowering of polymer concentration compromises resolution of smaller biomolecules. For example, optimal resolution of small DNA molecules requires an increase of derivatized agarose concentration to up to 8–9% polymer dry weight (Dumais and Nochumson, BioTechniques, 5 (1987) 62). It should also be noted that improved resolution of proteins is achievable by first partially depolymerizing agarose and then preparing a gel having the polymer content of around 5–6% (Nochumson et al, PCT/US90/00184).

The belief that a gel of high polymer concentration is necessary for resolution of small biomolecules is supported by a theory based on the extended Ogston model of gel electrophoresis. This model considers a gel as a random network of fibers and states that the electrophoretic mobility of a macromolecule is proportional to the volume fraction of the pores of the gel that the macromolecule can enter (Rodbard and Chrambach, Proc. Natl. Acad. Sci. USA'65 (1970) 907–977 and Tietz, Adv. Electrophoresis 2 (1988) 109–169). The model also postulates that there is no contact between migrating molecules and gel fibers. The measured electrophoretic mobility, μ, can be related to the free mobility in solution, μ_o, of a migrating molecule with radius R, as well as to the gel percentage T, total length of the gel fibers, **1'**, and the fiber radius, r:

$$\log \mu = \log \mu_o - \pi 1'(r+R)^2 T \times 10^{-16} \quad (1)$$