

DNA and RNA, peptides, proteins, and other biological materials commonly separated using electrophoresis techniques.

For the purposes of the present invention, the term “gellan gum” refers to a family of related carbohydrate polymers produced by *Sphingomonas* bacteria (previously identified as *Pseudomonas*) and would include gellan gum produced by genetically engineered bacteria and chemically modified gellan gums.

For the purposes of the present invention, the term “oligonucleotides” refers to chains of one or more nucleic acids and derivatives thereof. Examples of oligonucleotides include: DNA (both single and double stranded), RNA, etc. For the purposes of the present invention, the term “polypeptides” refers to molecules including two linked amino acids and derivatives thereof.

For the purposes of the present invention, the term “size-separation property modifying polymer” refers to polymers that can be incorporated into the gel of the electrophoresis medium to alter the size-separation properties of the electrophoresis medium of the present invention. Examples of size-separation property modifying polymers include: hydroxyethyl cellulose, dextran, ficoll, polyethylene oxide, polyacrylamide, etc.

For the purposes of the present invention, the term “reversibility” is used to refer to the ability of gellan gels to be returned to a liquid state.

For the purposes of the present invention, the term “zone” refers to a portion of an electrophoresis medium or gel contains substantially one biological material. Depending on the purity desired in a particular application of the present invention, there may be some degree of other biomolecules in a given zone in addition to the biological material which is to be recovered using the method of the present invention.

Description

The present invention provides a novel apparatuses and methods for the high-resolution separation and recovery of nucleic acids, proteins and similar biomolecules. The present invention also provides novel reversible electrophoresis gels.

The gels of the present invention are based on the carbohydrate polymer, gellan gum, that has a number of unique properties. Gellan gum forms gels under certain conditions and can be returned to a liquid solution by changing the conditions (reversibility). These gels are suitable for high resolution electrophoresis and the recovery of the separated nucleic acids and proteins. In general, the separation and recovery methods of the invention consist of the following steps: (1) performing electrophoresis on a sample of one or more biomolecules in a cross-linked gellan gel electrophoresis medium (2) removing one or more separated bands or sections (zones) of gel from the electrophoresis medium and (3) changing the condition in each of the removed zone to convert the gel in the zone into a liquid solution. In a preferred embodiment a removed zone of gel containing biological material may be contacted with a membrane, followed by changing the condition of the gel so that the gel returns to solution. The biological material left behind on the membrane may then be recovered by conventional means.

The present invention provides of novel electrophoresis gels that can be made to revert to a liquid solution by changing the ionic composition or the pH of the gels. The gels have many properties that make them suitable for the purification and manipulation of biomolecules. The gels of the present invention can be formed at low concentrations of

gellan gum. The conditions for causing the gels to revert to a liquid state that the delicate biomolecules contained in the gels are not damaged. The nucleic acid or protein can be separated from the the liquefied gellan gum by precipitation or other methods. The electrophoretic properties of the gels of the present invention can also be modified by the addition of polymers or compounds.

Gellan gum is a linear carbohydrate polymer produced by bacterial fermentation as described in U.S. Pat. Nos. 4,326,052; 4,377,636; 4,385,123 and European Patent No. 0 012 552, the entire disclosure and contents of which are hereby incorporated by reference. The carbohydrate polymer consists of repeats of tetrasaccharide units composed of two glucose sugars, a rhamnose and a glucuronic acid as described in O’Neil et al, “Structure of the Acidic Polysaccharide by *Pseudomonas elodato*” in *Carbohydrate Research* (1983), 124, 123–133 and Jansson et al, “Structural Studies of Gellan Gum, an Extracellular Polysaccharide Elaborated by *Pseudomonas elodato*” in *Carbohydrate Research* (1983), 124, 135–139. The gellan gum produced by fermentation has both O-acetyl and O-L-glyceryl 3-linked to glucose units. The acetyl groups can be removed during processing and the resulting materials are called low acyl gellan gums as described in Sanderson *Food Gels*, P. Harris (ed.) Elsevier Applied Science, (New York: 1990), 202–232. Commercially available low acyl gellan gums are called Gelrite and Phytigel.

Physical studies using X-ray crystallography have shown that crystals of gellan gum are intertwined, threefold left-handed parallel double helices. Ions are believed to promote the association of the intertwined double helix molecules in solution, resulting in gel formation as described in Rinaudo, “Gelation of Ionic Polysaccharides in *Gums and Stabilizers for the Food Industry*, Phillips et al. (ed.) (Oxford: 1988), 119, Chandrasekaran et al., “The Crystal Structure of Gellan” in *Carbohydrate Research* (1988) 175, 1–15 and in Chandrasekaran et al., “Cation Interactions in Gellan: An X-ray Study of the Potassium Salt” in *Carbohydrate Research* (1988), 181, 23–40.

Gellan gum has been used in food applications as a thickening agent and in plant tissue culture as described in Sanderson *Food Gels*, P. Harris (ed.) Elsevier Applied Science, (New York: 1990), 202–232. A procedure has recently been developed that removes the residual divalent ions in commercial preparations of gellan gum, as described in Doner and Dowds, “Purification of Commercial Gellan to Monovalent Cation Salts Results in Acute Modification of Solution and Gel-Forming Properties” in *Carbohydrate Research* (1995), 273, 225–233, and replaces them with a monovalent cation such as potassium or sodium. This procedure results in a chemically defined preparation that is soluble in water.

U.S. Pat. No. 5,143,646 describes the use of polysaccharide gel blends for stacking electrophoresis systems. This patent mentions using a large number of the polysaccharide gels which form polymers, including gellan gum for its mixtures. The gels in this patent are said to be “thermoreversible”, the gels can be liquefied by melting the polymer at high temperatures, and “pH reversible”, the gels can be liquefied by changing the pH to neutralize the charge on the gel. Because gellan has carboxy groups, liquefying gellan using the method described in this patent would require reducing the pH of the gel to less than 3 to protonate the carboxyl groups. However, at pH levels this low, any proteins or nucleic acids in the gellan could be denatured or broken down. With respect to temperature reversibility, for a typical gel described in U.S. Pat. No. 5,143,646, such as