

low melting point agaroses, the temperature must be raised to 65° C. to liquefy the gel. Just like excessively low pH, such high temperatures can degrade the biomolecules contained in a gel.

In contrast to the gels described in U.S. Pat. No. 5,143, 646, the gels in of the present invention do not require high temperatures of pH extremes. In one embodiment, the pH reversible gels cross-linked with a diamine can be liquefied (reversed) by the deprotonation of the diamine. Because the diamines used in the present invention preferably have pKs close to neutrality (pH 7), the gels containing them are reversible by changing the pH at around 7. At pH below 7 a gel will form and above pH 7 the gel will reverse to a liquid solution. The pH reversibility of the gels of present invention, in contrast to previous reversible electrophoresis gels, is based changing the state of a cross-linker for the gel and not the gel. For applications of the present invention where it is desirable to operate at pHs other than neutrality, the pH at which the gel liquefies can be adjusted by changing the chemical nature of the diamine used to form the gel.

The electrophoresis gels of the present invention can be formed using gellan gum in a wide range of concentrations, preferably from 0.03 to 2 grams per 100 mL. The gellan gum is dissolved in water or a buffer solution by mixing the gel into the solution. The solution can be heated to decrease the time necessary to dissolve the polymer particles.

The gellan gum can be cross-linked using either a divalent metal cation or a diamine. Where the cross-linking agent is a divalent metal cation, a variety of divalent metal cations can be used such as group IIA metal cations, such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, etc., or transition metal cations such as Zn<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, etc. The divalent metal cations are generally added to the gellan gum solution in the form of a metal salt. Typically the gellan gum polymer is mixed with the divalent metal cation cross-linking agent at a temperature above 60° C. and the solution is allowed to cool to form the gel. The divalent metal cation is preferably added to the electrophoresis buffer solution used in conjunction with the electrophoresis medium, preferably at a concentration of 0.1 to 10 mmol/L. Circulation of the buffer helps to prevent depletion of the divalent metal cation in the gel. Electrophoresis can be done in a variety of conventional formats including flat bed apparatus, vertical slab apparatus, tubes, and capillary tubes.

In the case of divalent metal cation cross-linked gels, after electrophoresis, the separated bands can be detected using a variety of means including specific stains or direct scanning of the gel. The bands containing the isolated solutes (biomolecules) can be recovered by a variety of means including adding a chelating compound specific for the divalent cation used to for the gel in solution form or attached to a solid substrate, such as an ion exchange resin.

Electrophoresis gels of the present invention employing diamine cross-linking agents can be formed in a similar manner to the gels using divalent metal cation cross-linking agent by mixing the diamine with the gellan gum polymer at a temperature above 60° C. Any buffer used in conjunction with the diamine cross-linked gels of the present invention should maintain the gel at a pH below the pK's of the amino groups of the diamine so that the amino groups are protonated.

In the case of diamine cross-linked gels, after electrophoresis, the separated bands can be detected using a variety of means including specific stains or direct scanning of the gel. The bands containing the isolated solutes (biomolecules) can be recovered by a variety of means

including adding a base to the gel either in solution form or attached to a solid substrate, such as an ion exchange resin. Once the pH of the gel is increased to the point where the amino groups of the diamine cross-linking agent are no longer full protonated, the gel reverts to a liquid solution.

In the case of either divalent metal cation cross-linked gels or diamine cross-linked gels of the invention, the properties of the electrophoresis gels of the present invention can be modified by the incorporation of additional polymers in the gel. For example, polymers can be incorporated in a gel to reduced the osmotic flow, and, thereby, increase the resolution of oligonucleotides and proteins by the gel.

The present invention will now be described by way of the following examples:

#### EXAMPLE 1

##### Properties of Gellan Gum Electrophoresis Gels and Modifications of Electroosmotic Flow by Additional Polymers Gel Formation, Electrophoresis, and Measurement of Electroosmotic Flow

Gellan gum (potassium salt ) was prepared using a deionization and precipitation procedure described in Doner et al., "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. The gellan gum powder was placed in a flask along with water and heated to boiling with stirring. The solution was stirred for 10 min to ensure that all particles of the polymer were dissolved. At this point additional polymers were added. Either the dry power or a concentrated liquid solution of the additional polymer was added and the solution stirred for an additional 10 min. In some cases the solution was heated to facilitate the dissolution of the added polymer. A concentrated solution (50 mmol/L) of either CaCl<sub>2</sub>, or 1,3 diamino-2-hydroxypropane (DAHP) was added so that the final concentration was 5 mmol/L. A solution of buffer (10-fold concentrated) was also added. The solution was stirred for an additional 10 min. The temperature of the solution was reduced to approximately 60° C. and the solution poured into the gel tray and allowed to solidify. A comb (16 teeth, 2 mm thick) was then suspended in the gel to form the sample wells. A flat bed submarine gel electrophoresis apparatus was used. The electrode buffer chambers were circulated by means of a peristaltic pump. The samples were diluted with a solution so the samples contained Ficoll 400,000 molecular weight (2% final concentration) or sucrose (5% final concentration), a trace of bromophenol blue, and the electrophoresis buffer. The samples were loaded into wells and the electric field applied. Measurement of electroosmotic flow in the gels was determined by measurement of the mobility of cyanocobalamin.

The buffers used for the gellan gum electrophoresis are shown in Table 1 below:

TABLE 1

Buffers Used for Gellan Gum Gel Electrophoresis		
Buffer	Composition	pH
TB	0.045 mol/L tris(hydroxymethyl)aminomethane (Tris) and 0.045 mol/L boric acid	8.5
TA	0.04 mol/L TRIS and 0.1 mol/L acetic acid	8.3