

Still another known sieving gel is SeaPrep® hydroxyethyl (derivatized) agarose, a product of FMC Corporation, BioProducts Group, Rockland, Me. 04841 U.S.A. There is a disclosure of the use of this product for sieving in electrophoresis by Nochumson, S. in *Electrophoresis* '81, 213-218, Allen & Arnaud (eds.), W. de Gruyter & Co. (pub.), New York (1981).

Other known sieving gel compositions include the mixture of agarose with hydroxyethyl cellulose as disclosed by Perlman, Chikarmane, and Halvorson in "Improved Resolution of DNA Fragments in Polysaccharide-Supplemented Agarose Gels", *Analytical Biochemistry*, 163:247-254 (1987).

This invention affords [1] novel polysaccharide resolving gel compositions; [2] novel "gel systems" comprising the foregoing novel and/or known polysaccharide resolving gel compositions electrophoretically associated with discontinuous polysaccharide stacking gels; and [3] kits comprising combinations of premeasured dry powder and/or premixed hydrogel resolving gels and discontinuous stacking gels, all of the foregoing optionally containing appropriate buffer systems. The resolving gels of this invention are particularly characterized by being capable of forming gels with improved sieving and resolving properties which can be prepared in high concentrations and without the handling problems normally presented by increasingly viscous casting solutions. Resolving gels and resolving-stacking gel systems have known utility in gel electrophoresis.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients, parameters, or reaction conditions used herein are to be understood as modified in all instances by the term "about".

As used herein the term "discontinuous" refers to a differential between a resolving gel and an associated stacking gel as to at least one of: gel pore size or polymer composition or the respective associated buffers' ionic strength, ionic composition, or pH. It is such a differential (discontinuity) that permits the stacking gel system of this invention to operate in the manner described herein. As is known in the electrophoresis art, there usually must be a second discontinuity between the electrode buffer and the buffer of the gel used. This second discontinuity is not part of this invention and is not included within the term "discontinuous" except where otherwise stated.

As used herein, the term "polysaccharide" refers to a native or derivatized thermoreversible and/or pH-reversible hydrogel-forming polysaccharide preferably selected from among: agar, agarose, curdlan, gellan, konjac, pectin, pullulan, and to a lesser degree alginate and carrageenan, and the like; as well as thermoreversible or pH-reversible hydrogel combinations of any of the foregoing with either another polysaccharide or a non-polysaccharide polymer which associates firmly with the gel-forming polymers so that it does not significantly dissociate under electrophoretic conditions. (Linear polyacrylamide does not associate firmly with the gel-forming polymers of this invention.)

In contrast to the present invention, the polyacrylamide gels of the prior art are known as neither pH- nor thermo-reversible. The hydrogel compositions and gel systems of the present invention specifically exclude acrylamide and do not require cross-linking or polymerizing agents.

The gels of this invention can be used in vertical, horizontal, and inverted casting formats using standard

equipment and buffers, and it is the increased ease of such casting without sacrifice of gel strength or resolving power that constitutes a most important advantage of this invention. Because they are thermoreversible and/or pH-reversible, the resolving and (separated) stacking gels of the present invention can be remelted after electrophoresis, enabling quick sample recovery and possible gel recovery and further gel utilization.

[I] In a first embodiment this invention affords a resolving gel composition comprising, preferably consisting essentially of, one or more (preferably two or more, more preferably two) polysaccharide hydrogels, at least one of which has been derivatized, and independently, at least one of which has been partially depolymerized sufficiently to reduce its viscosity to a degree that the casting ability of the resolving gel is improved, but not to the point where the resolving gel's strength is weakened beyond that required in electrophoretic procedures. It is possible to use as the hydrogels a mixture of a given derivatized hydrogel and the same gel after depolymerization. This may otherwise be stated as "depolymerized sufficiently to reduce the casting-effective viscosity of the resolving gel". The gel component polymers (when more than one is present) are physically blended (mixed) prior to formation of the hydrogel, and where the same polymer has been both depolymerized and derivatized, the order of such depolymerization and derivatization is not important. The inventive resolving gel preferably also comprises a suitable electrophoretic buffer, as will be discussed below.

The preferred polysaccharide for use in preparing the resolving gel composition of this invention is an agarose, and further disclosure herein will be stated in terms of agarose, although one or more of the other mentioned polysaccharides may be substituted therefore in a manner known in the art. Preferred agaroses for preparing the resolving gel composition are any native or derivatized agarose, examples of which include one or more of: native agarose; hydroxyalkylated agarose (more preferably hydroxy-C<sub>2-4</sub>-alkoxylated agarose, most preferably hydroxyethyl agarose); and dihydroxyalkylated agarose (more preferably dihydroxypropyl agarose, most preferably 1,2-dihydroxypropyl agarose), at least one of which has been depolymerized to reduce its viscosity at least to a casting-effective degree.

The most preferred 1,2-dihydroxypropyl agarose component of the inventive resolving gel composition is a known material which is prepared by reacting agarose with glycidol under alkaline conditions. It is isolated as a white powder or granules which form low melting gels with water. For a detailed description of its preparation, reference is made to U.S. Pat. No. 4,312,727—Shainoff.

In so far as this invention is a resolving gel composition per se, it should be noted that it excludes: (i) a single hydroxyethyl agarose hydrogel which has been subsequently partially depolymerized by gamma irradiation, [previously disclosed as useful for other purposes] and (ii) a mixture of three parts of (i) with one part of native agarose, [previously disclosed as useful for Polymerized Chain Reactions (PCR's)].

It is a critical aspect of the resolving gel compositions of this invention that they contain at least one agarose which has been depolymerized (i.e. degraded) sufficiently to reduce its viscosity. It is preferred that the viscosity of at least one agarose of the resolving gel composition is depolymerized until its viscosity is re-