

PURIFICATION AND USE OF GELLAN IN ELECTROPHORESIS GELS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application No. 60/427,988 filed Nov. 20, 2002, where this provisional application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is related to gellan purification, electrophoresis gel compositions containing purified gellan, and methods related thereto.

2. Description of the Related Art

Gel electrophoresis is currently employed for the separation of charged biological macromolecules such as proteins and nucleic acids. In gel electrophoresis, a mixture of charged species is resolved into its components owing to different mobilities of these species in a gel medium under an imposed electric field. The mobilities depend in large part on the characteristics of the charged species, including their net surface charge, which is affected by molecular size and shape.

Many types of gel material are suitable for use as the electrophoresis medium. The gel is often the determining factor in achieving a successful resolution of biological macromolecules, and accordingly the development of suitable gel materials has been the subject of intense research. Many gels are commercially available, and are typically composed of natural or synthetic polymers. Agarose is the most widely used natural material and polyacrylamide gels are the most common synthetic matrix.

In recent years, reversible gels have become commercially available and are increasingly popular for the purpose of preparative applications. In preparative applications, sections of the gel medium containing target biomolecules are reverted to the solution phase and the biomolecules therein are recovered by various means. For example, U.S. Pat. No. 5,143,646 describes the use of polysaccharide gel blends for stacking electrophoresis systems, wherein the gels are described as being "thermoreversible" and "pH reversible". In these particular reversible gels, the structure of the gel matrix is converted to a liquid form when the gel is subjected to heat or pH variation. Concerns have been raised, however, with regard to the conditions for reverting the "thermoreversible" or "pH reversible" gels to solutions. Problematically, the high temperature or specific pH (lower than 3 or higher than 9) needed for liquefying the gels can denature or otherwise alter the biomolecules contained in the gel matrix.

U.S. Pat. No. 6,203,680 discloses that gellan gum may be used as a reversible electrophoresis gel medium. Gellan-based gels have the advantage of being reversible under relatively mild conditions, and therefore address the concern about having the liquefaction conditions harm the biomolecules. Gellan gum can be liquefied under mild conditions because it can form a cross-linked gel in the presence of either divalent cations or diamines. In the case where divalent cations are used as the cross-linking agent, liquefaction of the gel may be achieved by adding a sequestering agent specific for the divalent cation. When diamine is used as the cross-linking agent, the pH of the gel is maintained at a value such that the amino groups of the diamine are protonated. The gellan gel reverts to a liquid solution when

the gel pH is adjusted so that the amino groups of the cross-linking agent are no longer protonated. This can be achieved under relatively mild pH conditions. Gellan-based electrophoresis gel can also be formed in the presence of cross-linked diamines that contain disulfide bonds. Gel formed in this way can be returned to solution using a reducing agent to break the disulfide bonds. A typical disulfide-containing cross-linker is cysteine dimethyl ester, also referred to as cystamine.

Gellan gum can be purified via a series of deionization and precipitation steps as described in Doner et al., "Purification of Commercial Gellan to Monovalent Cation Salts Results in Acute Modification of Solution and Gel-Forming Properties," *Carbohydrate Research* (1995), 273, 225-233. This purification procedure is time consuming and costly. Therefore, there exists a need in the art for gellan purified in an alternative and inexpensive way so it becomes economical to use gellan as a replacement for agarose.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method by which gellan, including commercial preparations of gellan, can be modified to render them particularly useful in electrophoresis gels. For instance, the present invention provides a method for purifying gellan where the method includes: (a) combining DNase and gellan, the gellan being contaminated with nucleic acid, thereby providing a mixture; and (b) maintaining the mixture of step (a) under conditions where the DNase degrades at least some of the nucleic acid, thereby providing purified gellan. Optionally, a size-separation property modifying polymer such as poly(ethylene oxide) may be added to the gellan or the purified gellan. In various optional embodiments, the gellan is contaminated with more than 100 ppm, or more than 10 ppm of nucleic acid, based on weight parts of gellan. The method of the present invention can reduce the nucleic acid contamination by 50% or more, e.g., to a level of less than 1 ppm nucleic acid based on weight parts of gellan. A DNase activating agent may be added to speed to rate of nucleic acid degradation, where sodium azide is a preferred DNase activating agent. Typically, the mixture of step (a) is maintained at about 30-45° C. for at least about 1 hour. Within less than about 24 hours, the nucleic acid has been essentially completely degraded. After the gellan has been treated to degrade the nucleic acid, the DNA may, optionally, be deactivated. For example, the treated mixture may be taken to a DNase inactivating temperature in excess of about 50° C.

The present invention thus provides gellan having very low levels of nucleic acid contamination. For example, the gellan may be in mixture with either no nucleic acid, or nucleic acid at a concentration of less than 10 ppm, or less than 5 ppm, or less than 1 ppm nucleic acid, where the ppm values are based on weight parts of gellan. These purified gellans are particularly useful in preparing an electrophoresis medium. For instance, the purified gellan may be in combination with a buffer composition suitable for maintaining said composition at a pH of 5-9. A buffer composition with imidazole or a salt thereof and boric acid or a salt thereof is a preferred buffer composition, where the buffer may additionally contain EDTA or a salt thereof. To form a suitable gel for electrophoresis, the purified gellan is in combination with a cross-linking agent. A preferred cross-linking agent is cystamine.

The present invention provides a superior method for treating gellan. Alternative methods require that the prepa-