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Rapid purification of commercial gellan gum to highly soluble and gellable monovalent cation salts[1]

Landis W. Doner

U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

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Commercial gellan gum contains divalent cation contaminants (mainly Ca^{2+} and Mg^{2+}) in levels sufficient to neutralize over one-third of its carboxyl groups. Consequently, in order to dissolve gellan gum in water, the mixtures must be heated to more than 90°C. This has prevented applications of gellan gum to such uses as the immobilization of viable enzymes and cells in beads. A rapid two-step method is described here to purify commercial gellan gum to the monovalent cation salts in an overall yield of 85%, through the intermediate free acid form. The gellan monovalent cation salts were highly soluble at temperatures as low as 5°C, and readily gelled upon exposure to solutions of divalent cations. Laboratory-scale preparations of 100 g of gellan monovalent cation salts were readily achieved in a day. Published by Elsevier Science Ltd

INTRODUCTION

Gellan gum is a bacterial (*Sphingomonas elodea*) exopolysaccharide consisting of tetrasaccharide repeating units →3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→ (Jansson *et al.*, 1983). A clarified form is marketed as a gelling agent for cell and tissue culture applications as Gelrite® by Kelco (a unit of Monsanto Co.). Gelrite® provides several advantages over agar for tissue culture applications, including optical clarity and greater gel strength at a given concentration (Sanderson, 1990).

The standard protocol for formation of gellan gels of various strengths is to heat autoclave temperature suspensions of 0.1 to 1.0% gellan in an aqueous solution containing divalent cations. Then upon cooling, gel formation occurs. Ca^{2+} and Mg^{2+} are present in levels of about 1% in commercial gellan, a level that neutralizes over one-third of the carboxyl groups in gellan. So a significant proportion of cations required for gel formation are already present in commercial gellan gum. Due to their presence the heating cycle is required for its dissolution. In order to provide uses for gellan gum analogous to those of sodium alginate, various researchers have attempted to facilitate dissolution of gellan gum at temperatures less than 90°C. The first approaches resorted to adding sequestrants such as EDTA, sodium citrate, or sodium hexametaphosphate (Baird & Shim, 1986; Camelin *et al.*, 1993) to commercial gellan gum. The dissolution of commercial gellan gum at moderate temperatures by these approaches resulted from the sequestering of divalent cations which contaminate commercial gellan gums. Gel formation was induced by addition to such solutions of sufficient levels of divalent cation to overcome the presence of sequestrant. The resulting milieu included divalent cation contaminants and sequestrant, and their presence would limit and perhaps prevent the use of such approaches to provide cultures or beads containing viable immobilized cells or tissues. In the reports (Baird & Shim, 1986; Camelin *et al.*, 1993), divalent cations were sequestered rather than removed. The routine procedures for removing divalent cations from soluble polysaccharides were not applicable.

We recently reported (Doner & Douds, 1995) a three-step procedure to purify commercial gellan gum, wherein divalent cations were removed. Monovalent salts (Li^+, Na^+, K^+, NH_4^+) of gellan were prepared as powders in 65% yields (Doner & Douds, 1995). The salts were freely soluble at temperatures as low as 5°C. Enzymes and cells in potassium gellanate solutions were converted to beads by dropping into stirred solutions of

[1]Mention of brand or firm names does not constitute an endorsement by U.S. Department of Agriculture above others of a similar nature not mentioned.

calcium or magnesium chloride. Potassium gellanate may also be used as a well defined gelling agent for producing transparent culture media. Viable embedded tissues, cells, and enzymes could be recovered after liquifying the resulting gels (Doner & Bécard, 1991). The advantages of using well defined and soluble gellan monovalent cation salts instead of commercial forms to which sequestrants were added to provide low temperature solubility are obvious. The purpose of this communication is to offer a streamlined purification procedure over that reported previously (Doner & Douds, 1995).

EXPERIMENTAL

Conversion of gellan divalent salts to free acid form

The Gelrite® form of gellan gum (20 g; Phytagel® from Sigma Chemical Co, St. Louis, MO or GelGro® from ICN Biochemicals, Cleveland, OH) was sprinkled into rapidly stirred deionized, distilled water (2 l) at room temperature. After the turbid mixture was heated to 60°C, 50 g of water-rinsed Dowex 50W-X8 (H^+, 20-50 mesh; Bio-Rad Laboratories, Melville, NY) cation exchange resin was added. The mixture was stirred at 60°C for 15 min, during which time the pH dropped from 6.1 to 2.2. The resin was allowed to settle out, and the turbid supernatant (maintained above 50°C) was then carefully removed from the resin by decantation. The resin was rinsed with 200 ml of 60°C water and the supernatant from decantation was combined with the original supernatant. The combined supernatants were held at 60°C.

Conversion of free acid form of gellan to monovalent cation form

The extremely turbid 60°C solution of the free acid form of gellan was converted to the desired monovalent cation form by gradual addition of 4 N NaOH, KOH, LiOH or NH_4OH. The gellan salt solutions became quite clear when the pH reached about 4.3, and addition of a further increment of alkali increased the pH to between 7 and 8. Stirring was continued at 60°C for an additional 5 min. The trace of turbidity was removed by vacuum filtration through several layers of fine-mesh cheesecloth, while maintaining the temperature above 50°C. The filtrate was poured into 2 volumes of rapidly stirred 2-propanol, and a white fibrous precipitate formed. The purified gellan was recovered on a no. 20 (850 μm) stainless steel sieve (Cu was detected in product recovered on brass sieves). Excess 2-propanol was squeezed from the product, and after air drying for 1 h, it was dried to constant weight in a vacuum oven at 40°C. The material was converted to a powder with a conventional chopper–grinder, and purified gellan was recovered in an overall yield of 85%. Elemental analysis by ICP were conducted by Galbraith Laboratories, Inc. (Knoxville, TN).

RESULTS AND DISCUSSION

Commercial gellan gum was purified to monovalent cation salts in two steps with a yield of 85%. Our earlier process (Doner & Douds, 1995) involved an additional step and a 65% yield was obtained. The initial step in both procedures involved heating to 60°C aqueous suspensions of commercial gellan gum with a cation-exchange (H^+) resin. A rapid exchange of protons for the cations contaminating commercial gellan gum resulted. In our earlier procedure, the cation exchange resin was then removed by vacuum filtration. Nearly one-quarter of the gellan gum was lost by filtration with the resin. In addition to loss of yield, loss of this portion of gellan gum made the filtration step cumbersome. This was due in part to the fact that it was necessary to maintain the filtrate temperature above 60°C before proceeding to the next step in order to prevent the mixture from gelling. In the modified process reported here, the liquid portion containing gellan gum in the protonated form was decanted from the cation-exchange resin.

The turbid suspensions of the acidic form of gellan were converted to the desired salt by addition of KOH or another monovalent cation hydroxide. Near the equivalence point the turbid material was largely dissolved and the solution quite clear. The mixture was clarified by filtration prior to precipitation of purified potassium gellanate with isopropanol. In Table 1 it is shown that this rapid two-step Phytagel® purification scheme (H^+ resin treatment, alkali addition) resulted in the removal of nearly all of the Ca^{2+} and Mg^{2+}. Other trace contaminants were also likely removed, including those that co-precipitated with gellan gum during commercial production upon addition of filtered culture media to alcohol.

In our original report (Doner & Douds, 1995) we needed to reduce phosphorus levels (0.1–0.2%) in the commercial gellan gum that we used as a culture gelling

Table 1. Levels (%) of major cation contaminants and phosphorus in Phytagel®, in potassium gellanate purified with Dowex 50W-X8 (H^+) resin alone, and with both Dowex 50W-X8 (H^+) resin and Dowex 1 X8 (Cl^-) resin[a]

Element	Phytagel®	H^+ resin treatment	H^+ and Cl^- resin treatment
Ca	1.2	0.04	0.06
Mg	0.39	0.004	0.01
K	1.6	3.39	3.43
P	0.21	0.09	0.02

[a]See Doner & Douds, 1995 for experimental details of Cl^- resin treatment.

agent. Phosphate inhibited colonization of plant roots by vesicular-arbuscular mycorrhizal fungi and it could not be tolerated. To remove phosphate, the pH 7–8 solution of potassium (or other monovalent cation) gellanate from step two was stirred at 80°C with an anion-exchange (Cl^-) resin (Doner & Douds, 1995). Solid potassium gellanate was precipitated from isopropanol after removing the resin by filtration. Over 90% of phosphorus was removed (Table 1) by using this third step. Where these levels of phosphate would be of little concern the anion-exchange may be eliminated. In Table 1 it is shown that over one-half of the original phosphate in commercial gellan gum was removed without anion exchange.

The gels prepared from gellan gum monovalent salts have comparable strengths to those prepared from conventional gellan gum. During the resin (H^+) treatment of commercial gellan gum the pH dropped to 2.2. At temperatures of 70°C or higher, sufficient depolymerization of the polysaccharide occurred so that the material no longer gelled with divalent cations. The purified gellan salts offer significant advantages over the commercial gellan gums. Most importantly, they are freely soluble in water at low temperatures. This allows immobilization of heat-labile materials into beads by adding solutions or suspensions of tissues, cells, enzymes to stirred solutions of divalent cations. Other advantages of the new form of gellan are defined composition, clarity, superior gel strength and the ability to dissolve the gels under mild conditions (Doner & Bécard, 1991) in order to recover viable materials for further analysis.

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