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Enhanced gelation properties of purified gellan gum

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ABSTRACT

Gellan gum is a hydrogel-forming polysaccharide when combined with monovalent or divalent cations such as sodium, magnesium, potassium or calcium. Commercially, gellan gums are sold with trace amounts of these cations, which have been proven to affect the gelation and mechanical properties of the resultant hydrogels. A new method based on impedance analysis for determining the gel transition temperature of purified and un-purified gellan gum is presented. The sodium salt form of gellan gum is shown to have lower dissolution and gel transition temperatures.

1. Introduction

Gellan gum is a anionic polysaccharide hydrogel-forming polymer produced from the bacteria *Sphingomonas elodea*.[1] Structurally, it comprises a tetrasaccharide repeat unit of two β-D-glucoses, one β-D-glucuronate, and one α-L-rhamnose[2] (Fig. 1). Gellan gum is available commercially under the trade names Gelrite™ and Kelcogel™ in 'high acyl' and 'low acyl' forms with the high acyl form being the native state.[3] The low acyl gellan gum is prepared via alkali treatment of the native gellan gum and is distinctively different in its gelation behaviour and mechanical properties[4]—high acyl gellan gum will form a gel upon cooling from 65 °C creating a flexible, soft hydrogel while low acyl gellan gum will form a gel upon cooling below 40 °C creating a rigid and brittle hydrogel.[5]

In recent years, low acyl gellan gum has become an attractive biopolymer for applications in tissue engineering as a cellular scaffold because it resembles the natural extracellular matrix (ECM) and is bio-inert.[6–8] Gellan gum has also been used as an injectable and printable matrix for cellular therapies and 3D tissue scaffold fabircation.[9–11] There is therefore potential for gellan gum based materials to be used for computer aided tissue engineering.[3,12]

Gellan gum, like many anionic polysaccharides forms a physical gel by undergoing a random coil to double helix transition upon cooling.[2] Stronger gels are formed if cations are present during the sol-gel transition.[2] In this case, divalent cations form particularly strong gels through the aggregation of helixes and monovalent cations form intermediate strength hydrogels through electrostatic interactions with carboxylate groups.[2] The presence of divalent cations also inhibits the ability of the un-hydrated gellan gum to become hydrated.[13] In the food industry, it is common practice to add calcium sequestrants (citrates and phosphates) to water to improve the ability of low acyl gellan gum to be hydrated.[14] The presence of cations in commercially provided gellan gum is ordinarily minimal and may not impede their use in food and pharmaceutical applications. However, very small amounts of calcium present in commercial gellan gums may still affect the more sophisticated chemistries used to modify gellan gum for tissue engineering applications.[3] Calcium may also affect the gel transition temperature so significantly that it precludes it from being utilised in rapid prototyping technology.

A method for the rapid purification of gellan gum was established two decades ago which employed an ion-exchange resin to capture the cations present in commercial gellan gum.[4,13] They reported that after purifying the gellan gum of divalent cations, the acid form gellan gum could be converted to a monovalent salt using a corresponding hydroxide salt. Sodium or potassium gellanate salts were able to be hydrated at much lower temperatures and formed gels of comparable strengths to un-purified gellan gum hydrogels.[4,13]

The research reported herein elaborates on Doner's[4,13] purification method, and provides quantitative information regarding the concentration of sodium, magnesium, potassium and calcium ions before and after purification; the temperature of hydration and gel

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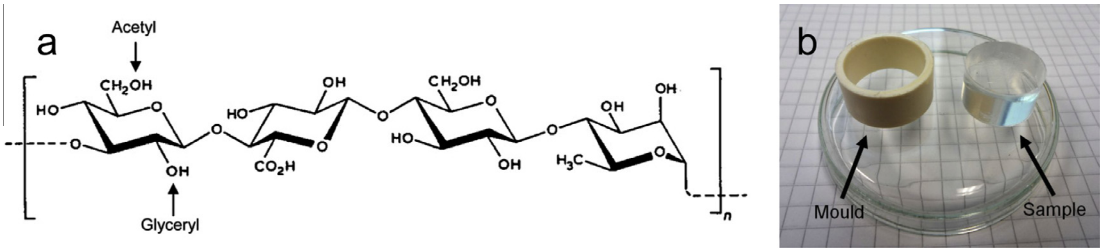

Figure 1. (a) The tetrasaccharide repeating unit of acid-form gellan gum. When deacylated, the indicated acetyl and glyceryl units are removed. (b) Photograph of the PVC mould and a typical gellan gum hydrogel sample used for mechanical testing.

transition temperature; and the mechanical properties of hydrogels prepared from purified and un-purified gellan gum solutions.

In this paper, we present a new method based on impedance analysis for determining the gel transition temperature of purified and un-purified gellan gum. Rheological and mechanical compression results are reported.

2. Materials and methods

All reagents used in these experiments were of AR Grade or better with inorganic contaminants present at 0.01% or lower. Deionised water (DI water) was prepared using a combination of reverse osmosis and ion exchange filter (Millipore, Australia) to a resistivity of 18.2 MΩ cm. All glassware was soaked in 10% (v/v) hydrochloric acid (Ajax Finechem, Australia) for 24 h prior to use to minimise contamination of the purified products and also to minimise contamination during inorganic elemental analysis. Purified gellan gum was prepared from low acyl gellan gum (GELZAN-CM, Lot #1/1443A, CP Kelco, Singapore, $pK_a = 3.5 \pm 0.2$, molecular weight ~200 kDa, 0.2% remaining acyl substituents) which was generously gifted from CP Kelco.

2.1. Moisture content

The moisture content of un-purified gellan gum was determined using an infrared moisture determination balance (AD-4712, A&D Company Ltd, Australia). A 5 g sample of gellan gum was heated on the balance to 80 °C and weighed periodically until a steady mass was attained.

2.2. FTIR spectroscopy

FTIR spectroscopy of dried samples was performed using a diamond attenuated total reflectance spectrometer (IRAffinity-1, Shimadzu, Japan) with 2 cm^{-1} resolution and Happ–Genzel apodisation.

2.3. Purification of gellan gum

Gellan gum (3 g) was dissolved in 300 mL of DI water at 80 °C whilst being stirred by an overhead mixer (RW 20, IKA, Australia) for 10–15 min at 300 rpm. Once dissolved, the gellan gum solution was cooled to 60 °C before adding 8 g of cation exchange resin (50WX8 DOWEX, Sigma Aldrich, USA). The mixture was stirred for 30 min at 60 °C before stirring was stopped and the resin was allowed to settle (pH = 2.2). The supernatant was then decanted and filtered (grade 165, Filtech, Australia) into a chilled reservoir containing 300 mL of 2-propanol (Ajax Finechem, Australia) with rapid stirring whereupon fibrous agglomerates of acid-form gellan gum (A-GG) precipitated. The A-GG was recovered from the 2-propanol solution using vacuum assisted filtration and freeze-dried (Alpha 1-2LDplus, Christ, Germany) for 48 h to remove residual water and 2-propanol.

The purified gellan gum sodium salt (Na-GG) was prepared in the same manner described above excepting that the supernatant, after being decanted, was neutralised with ~10 mL 0.4 M standardised NaOH solution until the pH of the solution reached 7.4 (826 pH-Mobile pH meter, Metrohm, Australia). After being neutralised, the supernatant was precipitated into 2-propanol and freeze dried as previously described.

2.4. Atomic absorption spectroscopy

Atomic absorption spectrometry of gellan gum samples was performed using a flame atomisation atomic absorption spectrometer (flame-AAS, Spectra AA 220FS, Varian, Australia) with hollow cathode lamp light sources for sodium (589.6 nm), potassium (769.9 nm), calcium (422.7 nm) and magnesium (285.2 nm), and an air/acetylene oxidant/fuel mixture.

Samples, blanks and calibration standards were prepared with a 5% (v/v) sulfuric acid matrix with CsCl (Sigma Aldrich, USA) added as ionisation suppressant. Calibration standards were prepared from certified multi-element standard solution (Lot #A2-MEB2366019, Inorganic Ventures, Australia). Samples were digested prior to analysis as follows: Approximately 1.00 g of sample was weighed and transferred quantitatively into a 150 mL Erlenmeyer flask. 5.0 mL of concentrated sulfuric acid (Ajax Finechem, Australia) was then added, followed by heating until the solids dissolved and the solution turned dark brown and started to fume. Hydrogen peroxide (30% v/v, Ajax Finechem, Australia) was then added drop-wise until the solution turned clear. The solution was then allowed to cool to room temperature (21 °C) before 16 mL of CsCl solution (12.6 mg/mL) was added. The solution was then diluted to 100 mL with DI water, mixed thoroughly, and measured within the day.

2.5. Gel transition temperature

The gel transition temperature for solutions of GG and Na-GG was assessed using a custom-designed electrical impedance instrument as well as a rheometer (Physica MCR-301, Anton Paar, Australia) in rotation mode. For electrical impedance analysis, hot gel solutions were poured into rectangular plastic troughs (1 cm × 1 cm × 4.5 cm) with reticulated vitreous carbon foam electrodes (foam structure with 20 pores per inch, relative density 3% or void volume 97%, resistivity 0.323 Ω cm, ERG Aerospace, USA) placed at either end such that the distance between the electrodes was 2.5 cm. The impedance analysis was performed by applying 1 V peak voltage and alternating current signals from a waveform generator (U2761A, Agilent, USA) to a circuit comprised of the gel sample cell and a 10 kΩ resistor in series while the temperature was measured simultaneously with a digital thermometer probe (Jaycar Electronics, Australia). Impedance across the gel