Research Article

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Gel point determination of gellan biopolymer gel from DC electrical conductivity

Abstract: Gellan is an anionic bacterial polysaccharide, which in aqueous solution dissociates into a charged gellan polymer molecule containing carboxyl ions and counter ions and forms thermoreversible gel under appropriate conditions. In this study, we investigated the effect of polymer concentration, the concentration of added monovalent metallic ion, and temperature on the DC electrical conductivity of the gellan. Results suggest that the DC conductivity decreases with the increasing polymer concentrations and the added monovalent metallic ions. Such a decrease in DC conductivity can be attributed to the reduction of the mobility of counter ions due to the increase in the crosslinking density of the gellan network. DC conductivity of gellan gels was increased with temperature, which is interpreted as the dissolution of physically cross-linked networks, thus increasing the mobility of counter ions. The behavior of temperature variation of DC electrical conductivity reveals an abrupt change at a specific temperature, which can be considered a way to determine the gel point or sol–gel transition temperature $T_c$ of this thermoreversible biopolymer gel. This result agrees with that of rheological measurements where the viscosity showed a similar trend with temperature and diverges to infinity at the temperature close to $T_c$.

Keywords: gellan gum, biopolymer gel, sol–gel transition, DC conductivity, monovalent ions

1 Introduction

Gellan is a negatively charged microbial polysaccharide, which in aqueous solution dissociates into a charged gellan polymer molecule containing carboxyl ions and counter ions and forms a thermoreversible gel. The fermentation of microorganism Sphingomonas elodea produces gellan (1). The most readily available gellan is in a deacetylated form with a tetrasaccharide repeating unit containing one carboxylic group (2). Owing to carboxyl groups’ possession in the repeating unit, the gelation of gellan is profoundly enhanced by cations in aqueous solutions. The monovalent counterions (cations) are responsible for screening electrostatic repulsion between adjacent molecules and promoting association, while the divalent ions facilitate the ionic bonding between two carboxyl groups. It is well known that gellan molecules adopt random coil conformation at high temperatures like 80°C. On cooling, cation-induced coil to double helix conformational change occurs, which is followed by a cation-mediated side-by-side helix–helix aggregation, leading to a three-dimensional network (3).

Since the gelation mechanism of gellan is vital for its application, several groups have conducted a series of studies to explore its gel and sol properties, including the sol–gel transition. The conformational change upon heating or cooling is a thermoreversible process where the helix–helix aggregation is the physical crosslinking point of the gellan network and has been characterized extensively using a variety of experimental techniques including rheology (4,5), light scattering (6,7), circular dichroism (CD) spectroscopy (8), and NMR spectroscopy (9,10).

Gellan aqueous solution is a polyelectrolyte solution with a negatively charged gellan molecule containing carboxyl ions and metallic ions as counter ions. With the application of voltage, the electrical current arises from the motion of mainly counter ions and charged gellan gives ionic conductivity, which depends on the concentration of ions, mobility, type of charge carriers, and temperature. Temperature affects conductivity by...
increasing the ionic mobility and the solubility of polymers and salts. As the gellan changes state with temperature, it is reasonable to anticipate that the ionic conductivity of the counterions in the solution state will be significantly different from that in the gel state simply because the solvent is trapped in the polymer matrix. If it is the case, the change in DC electrical conductivity of the gellan solution may allow us to determine the sol–gel transition temperature or the gel point.

To scrutinize these issues, we aimed at investigating the effect of polymer concentration, the concentration of added monovalent metallic ion, and the temperature on the ionic conductivity of gellan gel by applying the DC electric field in both gel and sol states.

2 Materials and methods

2.1 Materials

In this experiment, potassium-type gellan powder was used as the main raw material. This commercial powder sample was obtained from San-Ei-Gen FFI Ltd, Osaka, Japan, and was used in this study without further purification. The metal contents present in the dry gellan were analyzed as Na = 0.42%, K = 5.03%, Ca = 0.37%, and Mg = 0.09% by a LIBERTY Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) system (Varian Inc., Palo Alto, CA, USA). Nanopure water was used for preparing the solution.

2.2 Sample preparation

2.2.1 Gellan sample without added salt

The gellan gum solution was prepared by mixing the commercial powdered sample with nanopure water and stirred using a magnetic stirrer at 80°C for 2–3 h (depending on the polymer concentration) to achieve a complete dissolution (11). The dissolution was recognized by the transparency of the solutions. In this way, four samples of gellan with concentration C_p = 0.5, 1.0, 2.0, and 4.0 wt% were prepared, from which two samples of gellan concentration C_p = 1.0 wt% and 4.0 wt% will be used to prepare samples with the added salt. The samples were then stored in a refrigerator and preheated to 80°C for the later use. The heated solution was transferred to the cylindrical sample holder, where a transparent gel was formed from the uniform solution at ambient temperature.

2.2.2 Gellan sample with added salt

To produce a gellan solution with the added salt, the stock gellan solution was taken with a double amount of the desired concentration. The stock solution was prepared according to the method discussed in the previous section. The stock gellan solution prepared was of concentration C_p = 1.0 wt% and 4.0 wt%. For biopolymer gelation, the alkali metal ion K⁺ is known to have the strongest ability for transparent gel formation among monovalent metal ions (12). Thus, KCl salt was used as the added salt in this study. KCl stock solution was also prepared with a double amount of desired concentration. The same amount of both stock solutions by weight was carefully poured into a precleaned dust-free vial to obtain the desired polymer concentration, C_p, with different salt concentrations, C_s. Prepared samples were once again stirred by a magnetic stirrer at T = 80°C for 1 h, and the uniform solution was poured inside a cylindrical sample holder. In this way, the gellan concentration of C_p = 0.5 wt% and C_p = 2.0 wt% were prepared, and it was chosen because at a concentration 0.5 wt% gellan forms viscous solution, and at 2.0 wt%, it forms an elastic gel. The concentration of the added salt was fixed at C_s = 0.01 M and 0.05 M. Thus, in total, four samples of gellan with the added salt of C_p = 0.5 wt% and C_s = 0.01 and C_s = 0.05 M, and C_p = 2.0 wt% and C_s = 0.01 and C_s = 0.05 M were prepared.

2.3 Method

2.3.1 Electrical measurements

DC electrical characterization was done using a Keithley 2401 source meter. The sample was put inside a custom-made sample holder of mixed alloy with an inner diameter of 1 cm, and the gap between the two flat plates is 0.05 cm. The two flat sides of the cylindrical sample holder contain two electrodes (Figure 1). The outer layer of the cylindrical holder was sealed tightly using Teflon tape to ensure that the sample does not get evaporated during heating. The source meter was connected with the two electrodes of the sample holder by a copper wire and a computer. After the necessary connection, the sample holder was put inside a temperature-controlled closed
chamber (Binder oven). The lab tracer 2.9 software was used to obtain the $I-V$ characteristics curves at different temperatures ranged from 30°C to 70°C.

### 2.3.2 Rheological measurements

Shear flow measurements were performed using RheolabQC (Anton Paar) at various temperatures with cylindrical geometry shear stress $\sigma(t)$ measured as a function of shear rate. A cylindrical geometry zero shear rate viscosity was calculated for the gellan samples at different temperatures. The shear rate varied from 0.01 to 100 s$^{-1}$ and was changed stepwise from 0.01 to 100 s$^{-1}$ over 10 min.

### 3 Results and discussion

#### 3.1 Effect of temperature on conductivity

DC electrical measurements were performed on gel samples, which were kept inside a heating chamber capable of changing temperature from 25°C to 300°C. The source meter connected to a computer allowed us to measure the current as a function of the voltage directly applied using the software. The electrical conductivity of any material is the ability of a material to carry the flow of an electric current (a flow of electrons), estimated from the current-voltage characteristic curve. According to Ohm’s Law, the most straightforward $I-V$ characteristic involves a resistor, which exhibits a linear behavior between the applied voltage and the resulting electric current for ohmic materials. Environmental factors such as temperature and material characteristics of the resistor can produce a nonlinear curve.

Figure 2 shows the $I-V$ characteristic curve for gellan solution ($C_p = 0.5$ wt%) and gellan gel ($C_p = 2.0$ wt%) without the added salt measured by a source meter at a particular temperature of $T = 40^\circ$C. A similar trend was observed for the four gellan samples with different polymer concentrations and the added salt concentration.

The electrical conductivity ($\sigma$) of the sample was found using Eq. 1:

$$\sigma = d/RA,$$

where $d$ is the thickness of the sample, $R$ the resistance found from the $I-V$ curve, and $A$ is the area of the sample in contact with the electrode. The electrical conductivity was determined for all gellan samples at different temperatures.

Figure 3 shows the behavior of DC electrical conductivity of gellan gels with polymer concentration $C_p = 0.5$ wt% and salt concentration $C_s = 0.01$ M and 0.05 M KCl as a function of temperature ranged from 30°C to 70°C. As shown in Figure 3a, initially, the conductivity is found to be increasing linearly with the temperature with a gentle slope; however, an abrupt change of conductivity is observed after a specific temperature $T_c = 35^\circ$C, and the conductivity increases with $T$ linearly.

![Figure 1: Sample holder.](image)

![Figure 2: The $I-V$ characteristic curve for gellan solution ($C = 0.5$ wt%) and gellan gel ($C = 2.0$ wt%) without added salt.](image)
with temperature for the gel double helices for physically cross linking points (inside the network and therefore show a very low conductivity). As the temperature increases, the cross-linking points (called junction zone made by the association of double helices for physically cross-linked biopolymer gel) start to dissociate, and therefore, solvent and counter ions start to flow inside the network. As a result, an increase in electrical conductivity with the increasing temperature is expected, which is shown in Figure 3a.

The gel point or the sol–gel transition temperature is a critical point for a chemically cross-linked system; however, such a critical point may not be observed for a physically cross-linked system due to the short lifetime nature of physical bonds.

Therefore, the DSC peaks and NMR peaks for physically cross-linked gel are broad and difficult to accurately find the gel point (13,14). Instead, the dynamic viscoelasticity can provide the determination of the gel point where both the storage and the loss modulus, $G'$ and $G''$, respectively, show a power-law behavior with angular frequency (15).

In this study, the temperature $T_c$ identified in Figure 3a, can be proposed as the gel point for a physically cross-linked thermos-reversible gellan gelling system. Figure 3b shows the dependence of $\sigma$ with temperature for the same gellan concentration sample but with the KCl concentration of $C_s = 0.05$ M. Similar behavior types were observed for this sample; however, the transition temperature was shifted to the higher temperature $T_c = 46^\circ$C. This result suggests that the addition of metallic ions (KCl) increases the gel strength of the sample as more and more helices are associated due to the presence of added potassium ions, which are responsible for screening the electrostatic repulsion between the gellan helices (10).

In addition, the polymer concentration of the gellan system was changed from $C_p = 0.5$ to $C_p = 2.0$ wt% with different added salt concentrations. Results suggest that the DC conductivity decreases with the increasing polymer concentrations and the added monovalent metallic ions. These results can be explained in terms of the reduction of the mobility of counter ions due to the increase in the crosslinking density of the gellan network. Figure 4 shows the change in conductivity $\sigma$ as a function of the temperature. Results suggests that a sudden increase in conductivity is detected at $56^\circ$C for $C_s = 0.01$ M and $T_c = 58^\circ$C for $C_s = 0.05$ M KCl. The transition temperature $T_c$ found from Figure 4 is between $55^\circ$C and $60^\circ$C and is in close agreement with the results obtained from X-ray scattering (SAXS) and DSC studies on the effects of salts on Na-based gellan (13,16). It is reported in (16) for $C_p = 2.0$ wt% and $C_s = 0.05$ M KCl that the transition temperature found from the endothermic peak temperature in heating DSC curves and the exothermic peak temperature in cooling DSC curves is at the range of 48–50°C. The slight discrepancy of 5–10°C in transition between the two studies can arise because of the use of a differently premodified gellan sample. Besides, it was also observed that with the increasing salt concentration, the transition temperature shifts toward higher temperatures. It has been reported in literature for physical gels like gellan gel that with the increasing salt concentration, the network within the gel strengthens, and therefore, it requires a higher temperature to break the network to transit into the sol state (10,15).

To compare the results obtained here, we performed rheological measurements using a rotational rheometer. The rheometer usually measures viscosity as a function of
the shear rate. Shear flow measurements were performed on the 0.5 wt% and 2.0 wt% gellan samples keeping the concentration of K⁺ ion constant for different temperatures.

Results show that the sample viscosity has a very strong non-Newtonian behavior. In our case, this may occur due to the disentanglement of chains or dissociations of double helices with an increasing shear rate. This flow behavior suggests that association among the chains is present in the solution even at the high temperature of 80°C. From the viscosity, η vs shear rate curve, zero-shear rate viscosity has been estimated by extrapolating η to zero shear rate for different samples at different temperatures.

In Figure 5, the viscosity η₀ of gellan samples was plotted as a function of T. Viscosity η₀ shows nearly similar kind of T dependence to that of conductivity vs temperature dependence for the gellan samples. At higher temperatures, all the samples show finite viscosity. This viscosity increases gradually with the decreasing temperature and diverges to infinity at their respective Tm.

This critical temperature Tm can be considered as a sol–gel transition temperature or the gel-point. Similar behavior has already been reported for iota carrageenan, which is a helix-forming polysaccharide (17,18). The Tc and Tm for the samples are presented in Table 1, which reveals that the two methods are complementary. A small discrepancy of about 3°C is observed between Tc and Tm, which is due to the physical nature of the crosslinks and possibly the little difference in the temperature history.

Therefore, DC electrical properties provide a way to observe the sol–gel transition behavior and identify critical sol–gel concentration for gellan by simply monitoring the change in conductivity as a function of temperature, which agrees well with the rheological study and with other methods such as rheology, X-ray scattering, and DSC (11,13–16).
3.2 Effect of cation concentration on conductivity

The effect of the increasing cation concentration on two different gellan concentrations is shown in Figure 6. The figure shows that the cation effect is quite different in the solution form at 0.5 wt% than gellan in the gel form at 2.0 wt%.

For 0.5 wt% (Figure 6a) with the increasing cation concentration, the conductivity was observed to decrease drastically from $2.6 \times 10^{-2}$ Sc m$^{-1}$ with no added cations to $5.13 \times 10^{-4}$ Sc m$^{-1}$ with 0.05 M added cations. The higher conductivity with no added cation (in the range of $10^{-2}$ Sc m$^{-1}$) can happen because of two types of conduction process, one is the Grotthuss mechanism also called hopping where protons move through an aqueous environment. It has been observed in various similar polyelectrolytes (19,20), and the other is due to dynamic behavior of polymer chain in gellan (21). Now, the gellan sample used in this experiment was premodified with counter ions. Thus, even without any added counter ions, the sample had 4.78% of K$^+$ ions. Therefore, gellan without any added cations and low polymer concentrations exists as a randomly coiled form in the aqueous solution.

As a result, the movement of counter ions in the aqueous solution was just like ions in the saltwater solution. Because of the adoption of the random coil conformation, it did not entrap the movement of ions. However, as the counter ion (K$^+$ ion) was introduced keeping the gellan concentration constant, the increase of K$^+$ ion shielded the repulsive interaction among the free polyelectrolyte chains and created a cross-linked helix conformation, which later formed a three-dimensional gel network (3). With the slow formation of a gel network with the increasing cation concentration, the mobility of ions is reduced and the conductivity decreases.

For 2.0 wt% (Figure 6b) of gellan with the increasing cation concentration, the conductivity was observed to have almost no effect but a slight increase. It suggests that as gellan was in the gel state at 2 wt% without any added cations, the slight increase in conductivity arises from the effect of the increase of the number of ions (22). Therefore, the different order values of conductivity in

### Table 1: Comparison of $T_c$ and $T_m$ obtained from electrical and rheological measurements, respectively

| Concentration | $T_c$ from electrical measurement | $T_m$ from rheological measurement |
|---------------|----------------------------------|----------------------------------|
| $C_p = 0.5$ wt%, $C_s = 0.05$ M | 46°C                             | 49°C                             |
| $C_p = 2.0$ wt%, $C_s = 0.05$ M | 59°C                             | 56°C                             |

Now, the gellan sample used in this experiment was premodified with counter ions. Thus, even without any added counter ions, the sample had 4.78% of K$^+$ ions. Therefore, gellan without any added cations and low polymer concentrations exists as a randomly coiled form in the aqueous solution.

![Figure 6: Plot of conductivity at $T = 30°C$ vs KCl salt concentration for (a) $C_p = 0.5$ wt% and (b) $C_p = 2.0$ wt%](image-url)
the gellan aqueous solution and gel can suggest a conformational change within the gellan network.

Another interesting comparison of the effect of temperature and cation concentration on gellan was observed in Figure 7. Figure 7a shows that with the increasing temperature for a constant salt concentration, the conductivity does rise but once the cation concentration changes, the effect of the increasing temperature is not that extreme compared to the increase of cations.

A similar observation was made in the case of gellan 2.0 wt%. Therefore, it suggests that out of the two parameter temperature and salt concentration, the change of conductivity significantly depends on salt concentration for gellan in the aqueous form. As for the gel form at 2.0 wt%, the effect of the cation is almost independent. Therefore, the results suggested that the conformational change with the change of gellan concentrations and added monovalent metallic ions could be identified from the conductivity study.

4 Conclusion

Gellan is an ideal polysaccharide to understand the gelation of many other polysaccharides such as agar, carrageenan, and xanthan, and the temperature is a key parameter to describe such gelation process. From this study, the sudden increase in conductivity with the temperature indicates the characteristic parameter of gelation – the gel point or gel–sol transition temperature can be used as an alternative method to determine the gel point or gel–sol transition temperature of a biopolymer thermo-reversible gel. Gel point has also been obtained from rheological measurements, which are in agreement with that of electrical measurements. Moreover, the concentration-dependent conformational change of gellan can also be determined using conductivity rather than rheology and other methods.

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