Colloid Polym Sci 277:727–734 (1999)

ORIGINAL CONTRIBUTION

**E. Miyoshi**
**K. Nishinari**

# Non-Newtonian flow behaviour of gellan gum aqueous solutions

Received: 8 December 1998
Accepted in revised form: 5 March 1999

E. Miyoshi
Division of Development
and Environment Studies
Osaka University of Foreign Studies
Minoo City, Osaka 562, Japan

K. Nishinari (✉)
Department of Food and Nutrition
Faculty of Human Life Science
Osaka City University
Sumiyoshi, Osaka 558, Japan
e-mail: nisinari@life.osaka-cu.ac.jp
Tel.: +81-6-6605-2818
Fax: +81-6-6605-3086

**Abstract** Rheological properties of gellan gum solutions with and without salt have been monitored using oscillatory measurements and steady-shear viscosity measurements. The steady-shear viscosity measurements indicated that gellan gum solutions showed a wide Newtonian plateau when gellan gum molecules took a coil conformation, and that the shear-thinning behaviour became more conspicuous with conformational change of gellan gum molecules from coiled to helical, and the range of the Newtonian plateau became limited only to very low shear rates. When gellan gum solutions showed rheological behaviour as a dilute or concentrated polymer solution, these systems obeyed the Cox–Merz superposition of steady-state viscosity and dynamic viscosity. As gellan gum solutions formed a weak gel, the Cox–Merz rule was not valid; however, the deviation from this superposition was less significant than that of xanthan gum solutions which also show weak-gel behaviour.

**Key words** Gellan gum · Helix · Coil transition · Sol-gel Transition · Salt · Rheology

## Introduction

Many hydrocolloids have always played a significant role in the field of food science and technology and have long been used as texture modifiers, emulsion stabilizers, water absorption agents, and gelling and thickening agents. Most industrial polysaccharides originate from seaweeds, cultivated plants or microbial sources [1, 2].

Gellan gum is a microbial polysaccharide produced by fermentation of the organism *Pseudomonas elodea*. It is composed of a tetrasaccharide ($\beta$-D-glucose, $\beta$-D-glucuronic acid, $\beta$-D-glucose and $\alpha$-L-rhamnose) repeat unit, containing one carboxyl side group [3, 4]. It is widely used in the food industry and in biotechnology as a gelling, texturizing, stabilizing, film forming, suspending and structuring agent [5]. Moreover, it forms a transparent gel with outstanding flavour release [6], which is resistant to heat and its gel strength is less dependent on pH than many other polysaccharide gels [5]. The gelation mechanism and sol–gel transition of gellan gum are influenced strongly by cosolutes such as salts [7–11], polyols or sugars [11–14]. Gellan gum can provide a wide range of gel textures by careful control of added salts, and so these gels can give the same texture as other polysaccharide gels or can create new textures. However, the flow properties of gellan gum solutions have not been studied so much.

Xanthan gum is one of the most intensively studied food polysaccharides, and the validity of the Cox–Merz superposition for xanthan gum was studied in order to compare dynamic viscoelastic behaviours with large deformation properties [15–18].

Gellan gum shows the disorder–order transition on cooling or on adding salts, which is analogous to that of xanthan gum, and a gellan aqueous solution can change its behaviour from a dilute polymer solution to a weak gel depending on the polymer concentration and the temperature.

In the present work, the rheological properties of gellan aqueous solutions were investigated by dynamic viscoelastic measurements, and viscosity measurements, the results were compared, and the validity of the Cox–Merz superposition was discussed.

## Materials and methods

The purified sodium-form gellan samples kindly supplied by Dr. Sanderson of the Kelco Division of Merck & Co., California, USA, used in previous works [19–23] were used in the present work. KCl and $CaCl_2$ used in the present study were extra-fine-grade reagents (Wako Pure Chemical Industries, Osaka, Japan), and were used without further purification.

The gellan gum solutions were prepared in the same way as described previously [19]. The concentration of the gellan gum solutions was varied from 1 to 3.5 wt%. For samples containing salts, the concentration of the gellan gum solutions was fixed at 0.5 or 1 wt% and the concentration of KCl in the solutions varied from 5 to 50 mM, and that of $CaCl_2$ ranged from 0.85 to 6.8 mM.

Mechanical spectra and thermal-scanning rheological measurements were performed within a linear viscoelastic regime with a dynamic stress rheometer from Rheometrics Co., N.J, USA, using a parallel-plate geometry of 50 mm diameter with radial grooves to avoid gel slippage [19, 21–23]. The details of the rheological measurements have been described previously [19].

The steady-shear viscosity was measured at various temperatures under conditions of steady shear on a RFSI fluids spectrometer from Rheometrics Co., N.J. USA, using a cone and plate geometry of 25 mm diameter, over a shear-rate range from 0.01 to 100 $s^{-1}$. The shear rate was changed stepwise from 0.01 to 100 $s^{-1}$ over 10 min.

## Results and discussion

### Steady-shear viscosity for gellan gum solutions in the absence of salt

Figure 1 shows the shear-rate dependence of the steady-shear viscosity for 1, 2, 3 and 3.5% gellan gum solutions without salt at various temperatures. The 1% gellan gum solution showed a flow behaviour close to Newtonian flow at 40 or 30 °C, and had a viscosity of about 10 mPas (at 40 °C) or of about 100 mPas (30 °C), nearly irrespective of the shear rate (Fig. 1a); however, the 1% gellan gum solution changed to shear-thinning behaviour at 20, 10 or 5 °C. The range of the Newtonian plateau at low shear rate gradually became narrower, and the viscosity became more shear-rate-dependent with decreasing temperature. Intermolecular entanglements disrupted by the imposed deformation resulted in new interactions at low shear rates, and so there was no overall change in the extent of entanglement, which corresponds to the Newtonian region in the flow curves [24]. When the rate of disruption becomes greater than the rate of formation of new entanglements, the onset of shear thinning may occur. The extent of re-entanglement decreases with increasing shear rate, and so the viscosity of the solution may gradually decrease [24]. A behaviour close to Newtonian flow was observed at 40 °C for the 2% gellan gum solution (Fig. 1b).

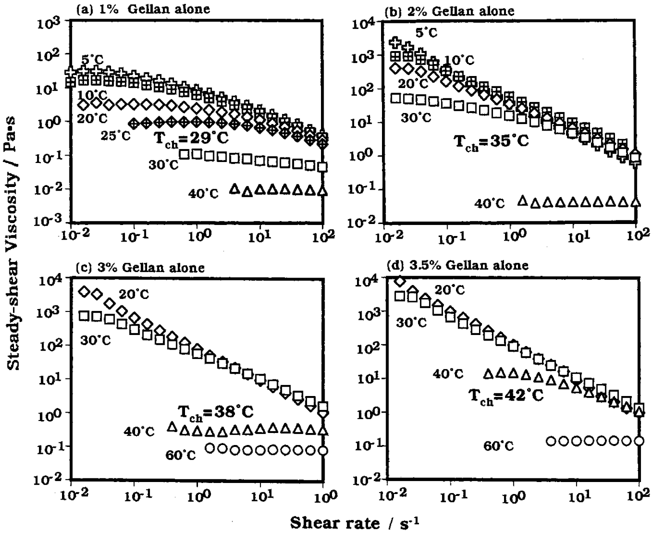


**Fig. 1a–d** Shear-rate dependence of viscosity for 1, 2, 3 and 3.5% gellan gum solutions without salt at various temperatures. (○) 60 °C; (△) 40 °C; (□) 30 °C; (◈) 25 °C; (◇) 20 °C; (⊞) 10 °C; (✙) 5 °C

As shown in previous studies [22, 23], $G''$ for the 1% gellan gum solution showed a steplike change ($T_{ch}$) at 29 °C in the thermal-scanning rheological measurement. This transition temperature was in good agreement with the characteristic temperature ($T_c$) where the molecular ellipticity $[\theta]$ at 204 nm showed a steep change in circular dichroism (CD) observed by another research group [25]. They used the same gellan gum sample and the same scanning rate [25]. A similar tendency was observed for gellan gum solutions of concentrations less than 1.0%. The reported characteristic temperatures determined by CD were 21, 24 and 27 °C for 0.32, 0.5 and 0.81% gellan gum solutions, respectively [25]. In the present work, the thermal-scanning rheology showed that $T_{ch}$ were 20, 22 and 26.9 °C for 0.3, 0.5 and 0.8% gellan gum solutions, respectively (data not shown). Therefore, it was suggested that gellan gum molecules changed from coil conformations to helical conformations at $T_{ch}$, where $G''$ showed a steep increase during cooling.

Steady-shear viscosity measurement indicated that gellan gum solutions tend to show more shear-thinning behaviour with the conformational change from coiled to helical because the helix may be more easily orientated along the shear flow than the coil. The range of the Newtonian plateau at low shear rates gradually becomes narrower with development of an ordered structure of the gellan gum solution.

For the 3 or 3.5% gellan gum solution (Fig. 1c, d) as well as the 1 or 2% solution (Fig. 1a, b), the flow curve showed a behaviour close to Newtonian flow above $T_{ch}$ (38 °C for a 3% solution or 42 °C for a 3.5% solution, respectively) while it changed to shear-thinning behaviour below $T_{ch}$. However, at lower temperatures where gellan gum formed an elastic gel, the steady-shear viscosity measurement could not be performed.

The stress as a function of shear rate for 1, 2, 3 and 3.5% gellan gum solutions without salt at various temperatures is shown in Fig. 2. Open symbols represent flow curves for these solutions with increasing shear rate from 0.01 to 100 $s^{-1}$, and closed symbols represent those with decreasing shear rate from 100 to 0.01 $s^{-1}$ after the increasing process of the shear rate. For all solutions, the stress increased linearly with increasing shear rate at temperatures higher than $T_{ch}$, and the yield stress seemed to be very small. The slope of double logarithmic plots of stress against shear rate at higher temperatures (1% at 40 and 30 °C, 2% at 40 °C, 3% at 60 and 40 °C, and 3.5% at 60 °C) was approximately 1, as in Newtonian flow. However, the yield stress was obtained by fitting the extrapolation to zero shear rate in the linear scale plot at relatively lower temperature ($<T_{ch}$) (2% at 5 °C, 3% at 20 °C, and 3.5% at 30 and 20 °C) (data not shown) and the stress showed no remarkable increase with increasing shear rate, i.e. it became markedly shear-thinning. For a 3.5% solution at 20 °C (Fig. 2d), the parameter $n$ in the Herschel and Bulkeley model $\tau - \tau_t = \kappa\dot{\gamma}^n$ approached zero, therefore this solution showed marked shear-thinning behaviour.

For 2, 3 and 3.5% solutions at sufficiently low temperatures (2% at 5 °C, 3% at 20 °C, and 3.5% at 30 and 20 °C), the flow curves showed a maximum stress at a fairly low shear rate and then the steady stress became slightly shear-rate dependent (open symbols). However, the flow curves for these solutions during the decreasing process of shear rate (closed symbols) showed no maximum stress. Moreover the difference between flow curves during the increasing and decreasing processes became larger with decreasing shear rate. For 2, 3 and 3.5% solutions at relatively high temperatures and 1% solutions at any temperature, the differences between flow curves during the increasing and decreasing processes were negligibly small, compared to those for 2, 3 and 3.5% solutions at sufficiently low temperatures. This can be explained as follows. As illustrated previously [19, 21–23], at any temperature from 30 to 0 °C, the mechanical spectra for a 1% gellan gum solution showed behaviour typical of a dilute polymer solution with $G' < G''$ throughout the accessible frequency range, and both moduli were strongly frequency dependent [26–30]. The frequency dependence of the moduli tended to $G' \sim \omega^2$ and $G'' \sim \omega$ at $30 \sim 15$ °C, which is a characteristic feature of dilute polymer solutions [31]. However, both moduli of this solution deviated slightly from this behaviour at 0 °C. Therefore, the individual helices of the gellan gum molecules were formed at a certain temperature ($T_{ch}$) during cooling, and then these helices gradually aggregated with decreasing temperature. This ordered structure, however, does not lead to gel formation because the number of helical aggregates is not sufficient to develop a continuous network throughout the whole space. On the other hand, for the concentrated gellan gum solutions ($>2$%), where the number of aggregated helices exceeds a critical value on cooling, the crossover temperature of $G'$ and $G''$ appeared at temperatures lower than $T_{ch}$ in the thermal-scanning rheology, as described in previous studies [22, 23]. This transition corresponded to the sol–gel transition, because these solutions changed to a weak gel at temperatures lower than the crossover temperature ($T_{sg}$), as described in previous studies [22, 23]. Therefore, the 2% solution at 5 °C, the 3% solution at 20 °C, and the 3.5% solution at 30 or 20 °C behaved as weak gels, and the flow curves for these stiff structures showed distinct maximum stresses at fairly low shear rates. It is difficult to understand that the stress decreased slightly with increasing shear rate. This might be attributed to one or both of the following factors. 1. These stiff structures (weak gels) might be ruptured at a certain shear rate at which the stress was a maximum. 2. Water exuded from the solution forms a thin layer between the cone (and/or plate) and the solution, and causes the slippage.

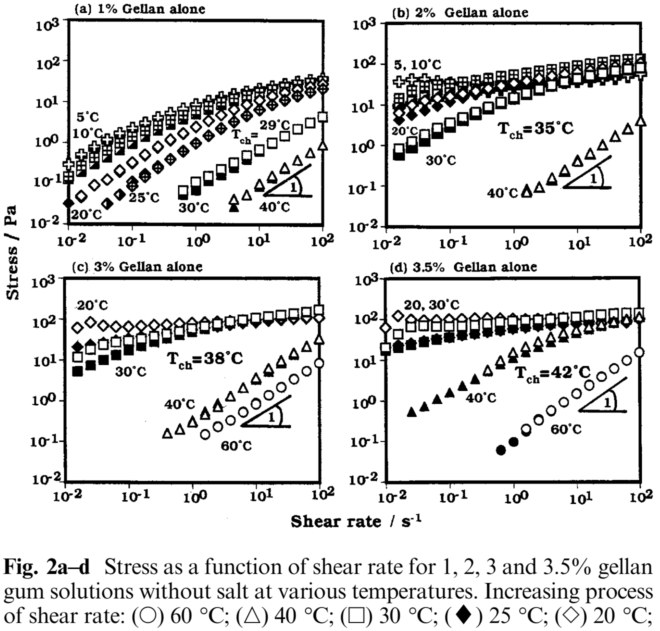


**Fig. 2a–d** Stress as a function of shear rate for 1, 2, 3 and 3.5% gellan gum solutions without salt at various temperatures. Increasing process of shear rate: (○) 60 °C; (△) 40 °C; (□) 30 °C; (◆) 25 °C; (◇) 20 °C; (⊞) 10 °C; (✙) 5 °C. Decreasing process of shear rate : (●) 60 °C; (▲) 40 °C; (■) 30 °C; (◈) 25 °C; (◆) 20 °C; (◪) 10 °C; (✚) 5 °C

After showing a slight decrease in stress, these solutions flowed and became strongly shear-thinning fluids whose stress was much less dependent on shear rate. After the increasing process of shear rate, weak gels would require enough time to recover their original properties when shearing is stopped, and so the shear stress in the flow curves for these systems during the decreasing process of shear rate became smaller than that during the increasing process of shear rate, especially in the lower shear-rate range.

These systems could not flow at shear stresses lower than the yield stress in the increasing process: however in the decreasing process, these structures have already been broken down by large deformation and flowed freely, and so the maximum observed in these systems at low shear rates in the increasing process could not appear. The distinction between weak gels and entanglement systems seems to be demonstrated using the Cox–Merz rule [27], as described later.

## Applicability of the Cox–Merz rule

It has been suggested that for solutions of entanglement systems such as xanthan gum [15–18], galactomannan [32] and gellan gum [33, 34], the steady-shear viscosity ($\eta$) and the magnitude of the complex viscosity [$|\eta^*| = (G'^2 + G''^2)^{1/2}/\omega$] superimpose closely at equivalent values of shear rate ($\dot{\gamma}/\mathrm{s}^{-1}$) and frequency ($\omega/\mathrm{rad\,s}^{-1}$), i.e. these solutions obey the Cox–Merz rule. If the mechanical spectra for the solutions show a weak-gel behaviour, $|\eta^*|$ is significantly larger than $\eta$ indicating that the weak-gel networks can survive small oscillatory deformation but may be ruptured by large deformation, and so these solutions tend to depart from the Cox–Merz superposition [18, 27].

Figure 3 shows the shear-rate dependence of the steady-shear viscosity $\eta$ and the frequency dependence of the absolute value of the complex viscosity $|\eta^*|$ for 1, 2 and 3% gellan gum solutions without salt at various temperatures, and the frequency dependence of the storage and the loss shear moduli for these solutions at various temperatures. The 1% solution behaved as a dilute polymer solution (with $G' < G''$ throughout the accessible frequency range, and both moduli were strongly frequency-dependent) even if gellan gum molecules took a helical conformation (Fig. 3d). The shear-rate dependence of $\eta$ (closed symbols), was in good agreement with the frequency dependence of $|\eta^*|$ (open symbols) at any temperature from 30 to 5 °C, which showed that the Cox–Merz superposition was well satisfied (Fig. 3a). The viscoelastic behaviour for the 2% gellan gum solution was that of a dilute polymer solution at 40 °C and a concentrated polymer solution at 30 or 20 °C (which showed a crossover of $G'$ and $G''$ at a certain frequency) (Fig. 3e), and this solution at these temperatures obeyed the Cox–Merz rule (Fig. 3b); however, at 5 or 0 °C, the 2% gellan gum solution changed to weak-gel behaviour (Fig. 3e), and so $|\eta^*|$ (open symbols) was slightly larger than $\eta$, especially at high shear rates (frequencies), indicating departure from the Cox–Merz superposition (Fig. 3b). For the 3% solution (Fig. 3c), the Cox–Merz superposition was satisfied when this solution behaved as a dilute polymer solution (at 60 or 40 °C) or a concentrated polymer solution (30 °C) (Fig. 3f), while there was no evidence of the Cox–Merz superposition when this solution showed weak-gel behaviour (at 20 °C) (Fig. 3f). These tendencies were similar to those of xanthan gum solutions; however, the departures from the Cox–Merz superposition seem to be more pronounced for xanthan gum [15–18] than for gellan gum in the present study. Xanthan gum shows the disorder–order transition on cooling or

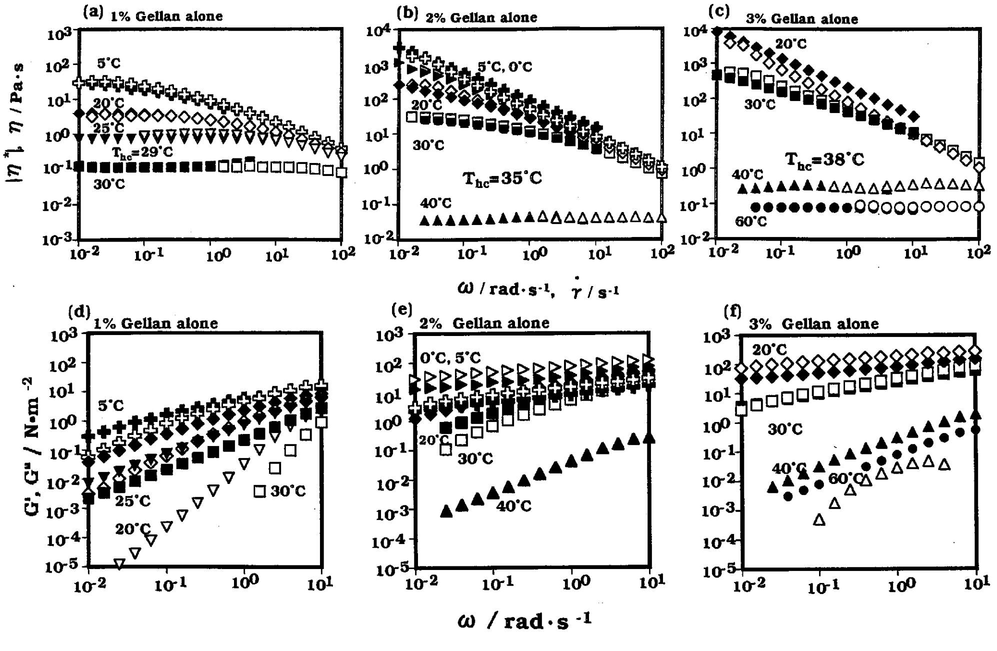


**Fig. 3a–f** Shear-rate dependence of the steady-shear viscosity $\eta$ and frequency dependence of the absolute value of the complex viscosity $|\eta^*|$ for 1, 2 and 3% gellan gum solutions without salt at various temperatures. *Upper figures* **a–c**: (○) $\eta$, (●) $|\eta^*|$, 60 °C; (△) $\eta$, (▲) $|\eta^*|$, 40 °C; (□) $\eta$, (■) $|\eta^*|$, 30 °C; (▽) $\eta$, (▼) $|\eta^*|$, 25 °C; (◇) $\eta$, (◆) $|\eta^*|$, 20 °C; (✙) $\eta$, (✚) $|\eta^*|$, 5 °C; (▷) $\eta$, (▶) $|\eta^*|$, 0 °C. *Lower figures* **d–f**: (●) $G''$, 60 °C; (△) $G''$, (▲) $G'$, 40 °C; (□) $G'$, (■) $G''$, 30 °C; (▽) $G'$, (▼) $G''$, 25 °C; (◇) $G'$, (◆) $G''$, 20 °C; (✙) $G'$, (✚) $G''$, 5 °C; (▷) $G'$, (▶) $G''$, 0 °C

on addition of salt [34], which is analogous to that of gellan gum: however, this transition temperature was significantly higher than that of gellan gum. Since for a polysaccharide such as xanthan gum, most reports investigating the validity of the Cox–Merz superposition have used the results obtained at room temperature (around 25 °C), it has been reported that the mechanical spectra of xanthan gum solution around 25 °C did not change to the behaviour of a dilute polymer solution except at quite a low concentration of polymers [18]. As is well known for biopolymers such as gellan gum or xanthan gum, even a subtle difference in molecular structure and molecular weight can lead to a significant difference in their rheological properties. Therefore, the reason why the difference between $|\eta^*|$ and $\eta$ of gellan gum showing weak-gel behaviour was markedly smaller than that of xanthan gum also showing a weak-gel behaviour seems to be related to the difference in molecular conformation and molecular weight.

For the present sample, the number-average molecular weight $M_\mathrm{n}$ for soluble tetramethylammonium-type gellan gum determined by osmotic pressure measurements was $5.0 \times 10^4$ at 40 °C and $9.5 \times 10^4$ at 28 °C [35]. Therefore, $M_\mathrm{n}$ at 28 °C was twice as large as that at 40 °C, which suggested the association of two molecules at 28 °C [35]. In the ordered state for the present sample (where gellan gum molecules took a double-helical conformation), the weight-average molecular weight $M_\mathrm{w}$ for soluble tetramethylammonium-type gellan gum determined by light-scattering measurements was $2.38 \times 10^5$ at 27 °C [36]. By comparison of $M_\mathrm{w}$ with $M_\mathrm{n}$ which was obtained in the ordered (helical) form, the present gellan gum has a polydispersity index of approximately $M_\mathrm{w}/M_\mathrm{n} = 2.5$, which corresponded to that of typical calculations ($M_\mathrm{w}/M_\mathrm{n} = 2$). The molecular weight of xanthan gum has been reported [15–18, 34] to be approximately $1 \sim 7 \times 10^6$, which is much higher than that of gellan gum. It has been reported [37] that both gellan gum and xanthan gum have significantly larger values of the Kuhn segment length than DNA (about 120 nm), and all the microbial polysaccharides reported in Ref. [37] have approximately the same Kuhn segment lengths, although the statistical variation of the Kuhn segment length for gellan gum was slightly larger than that for xanthan gum. From the chain stiffness drawn from static light-scattering data [37], it has been suggested that the Kuhn segments in xanthan gum behave like truly rigid rods, while those in gellan gum might have some flexibility. This fact might explain the differences in the rheological behaviour between xanthan gum and gellan gum mentioned previously. Graessley and Segal [38] have suggested that as the molecular weight of polystyrene becomes greater, the shear-thinning behaviour of polystyrene solutions becomes more conspicuous. Therefore, the ordered structure of xanthan gum may be more easily broken down along the shear flow, compared to that of gellan gum, because the molecular weight of xanthan gum is much higher than that of gellan gum. It has been reported that xanthan gum can form a weak gel at room temperature even at a low concentration [18]; however, the helices of gellan gum molecules cannot form a weak gel if the concentration is not high enough (>2.0%) and the temperature is not low (<7 °C for a 2.0% solution) [22]. It has been reported [33, 34] that at higher temperatures, gellan gum solutions with a random coil conformation obey the Cox–Merz rule, while at temperatures below the transition, they tend to depart from this rule, as in the case of xanthan gum. Since the gellan gum solution (<2%) in the present work behaved as a dilute polymer solution even at temperatures much lower than the coil–helix transition ($T_\mathrm{ch}$), the Cox–Merz rule was obeyed; however, the concentrated gellan gum solutions (≥2%) could behave as weak gels since sufficient aggregates of helices were formed at temperatures below the sol–gel transition temperature $T_\mathrm{sg}$, and so the Cox–Merz superposition was satisfied at temperatures higher than $T_\mathrm{sg}$ but failed at temperatures lower than $T_\mathrm{sg}$.

The flow behaviour for hyaluronate solutions of various concentrations has been reported [39, 40]. The critical concentration $c^*$ representing the transition between the dilute and the concentrated regime, and the critical shear rate ($\dot{\gamma}_\mathrm{c}$) corresponding to the onset of shear thinning have been reported. In the present work, $\dot{\gamma}_\mathrm{c}$ for gellan gum solutions of various concentrations was determined at 30 °C, because $\dot{\gamma}_\mathrm{c}$ could be determined neither at higher temperature at which the gellan gum solution showed flow behaviour close to Newtonian flow, nor at lower temperatures at which the gellan gum solution did not show experimentally the plateau at low shear rates.

The critical-shear-rate value ($\dot{\gamma}_\mathrm{c}$) of the onset of shear-thinning behaviour as a function of concentration for gellan gum solutions without salt at 30 °C is shown in Fig. 4. At this temperature, the $\dot{\gamma}_\mathrm{c}$ value decreased with increasing concentration of gellan gum; the concentration dependence of the critical shear rate was $\dot{\gamma}_\mathrm{c} \sim c^{-3}$, which was in good agreement with experimental results obtained by Fouissac et al. [39] and Mo et al. [40]. It has been suggested that in the semidilute regime for hyaluronate solutions with NaCl, the dependence of $\dot{\gamma}_\mathrm{c}$ on $M_\mathrm{w}$ and $c$ is given by $\dot{\gamma}_\mathrm{c} \sim c^{-3} M_\mathrm{w}^{-4.8}$ [39]. We will investigate the flow behaviour of gellan gum solutions using gellan gum with various molecular weights in the future.

## Steady-shear viscosity of gellan gum solutions in the presence of salt

Figure 5 shows the shear-rate dependence of the steady-shear viscosity for 0.5% gellan gum solutions containing

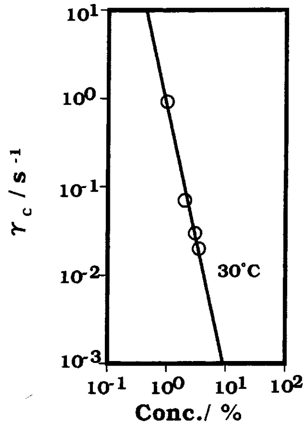
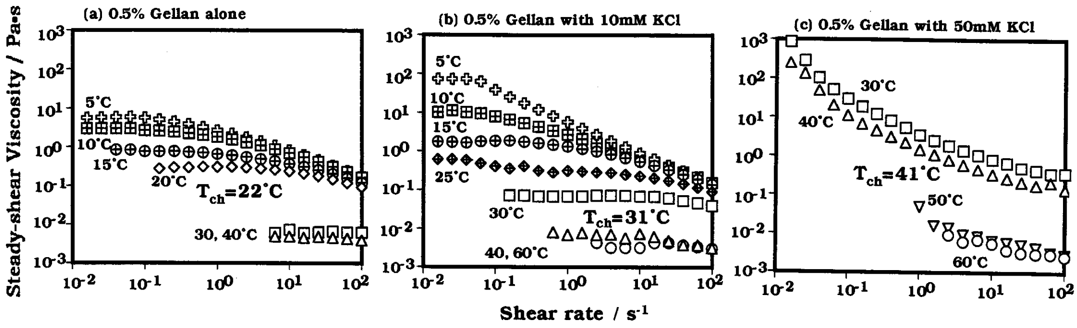


**Fig. 4** The critical-shear-rate value ($\dot{\gamma}_c$) of the onset of viscoelastic behaviour as a function of concentration for gellan gum solutions without salt at 30 °C

KCl of various concentrations at various temperatures. In the presence of salt, $T_{ch}$ determined by rheological measurement was in good agreement with $T_c$ determined by CD. The coil–helix transition temperatures $T_{ch}$ were determined to be 33, 39, 34.7 and 43.3 °C for 0.8% gellan gum solutions containing 20 mM NaCl, 50 mM NaCl, 20 mM KCl and 50 mM KCl (in the present work, data not shown), while $T_c$ were determined to be 32, 38, 34.5 and 42 °C for 0.81% gellan gum solutions containing 20 mM NaCl, 50 mM NaCl, 20 mM KCl and 50 mM KCl [25].

Since salt could significantly promote the formation of ordered structures in gellan gum [8–11, 33], in the present work, we chose a relatively low concentration of gellan gum (0.5%) in systems containing salt. By using dynamic viscoelastic measurements, $T_{ch}$ were determined to be 22, 31 and 41 °C for the 0.5% gellan gum solution without salt, with 10 mM KCl and 50 mM KCl, respectively (data not shown). A 0.5% gellan gum solution without salt (Fig. 5a) showing the shear-thinning behaviour had a wide quasi-Newtonian region even at much lower temperatures (10 or 5 °C) where it formed a significantly ordered structure. Upon addition of 10 mM KCl (Fig. 5b), the temperature at which the shear-thinning behaviour began to appear (gellan gum molecules formed an ordered structure) shifted to higher temperatures, compared to that without salt (Fig. 5a), and the Newtonian region at low shear rate became significantly narrower with decreasing temperature. However, at higher temperatures where the gellan gum solution behaved like a Newtonian fluid, the viscosity in the presence of KCl was slightly lower than that without salt (Fig. 5a). Therefore, in the absence of salt, the individual coils of gellan gum molecules (at higher temperatures) were expanded by intermolecular electrostatic repulsions between carboxyl groups in gellan gum molecules [8, 9, 19, 21–23, 41]. In contrast, the addition of salt screened the electrostatic repulsions and led to a reduction in the coil dimension [19, 21–23, 33, 42], hence the decrease in viscosity. At higher temperature such as 60 or 50 °C, the 0.5% gellan gum solution with 50 mM KCl (Fig. 5c) had similar values of viscosity to that without salt at 40, and 30 °C, and behaved like a Newtonian fluid. However, the solution with 50 mM KCl showed slight shear-thinning behaviour even at these higher temperatures. Therefore, in the presence of sufficient salt, gellan gum solutions became more inhomogeneous, and so these solutions did not seem to behave like a Newtonian fluid even at higher temperatures where most of the gellan gum molecules take a coil conformation. Upon addition of 50 mM KCl (Fig. 5c), a marked shear-thinning behaviour was observed even at a fairly high temperature such as 40 °C. Moreover, there was no evidence of Newtonian flow even at very low shear rates (the plateau at low shear rates could not be accessed experimentally), which indicated the formation of intermolecular networks by association of ordered polymers, as in the case of xanthan gum which was in an ordered, elongated conformation [33]. Therefore, it was suggested that in the presence of sufficient salt, the individual helices were significantly aggregated, and so gellan gum solutions formed a more ordered

**Fig. 5a–c** Shear-rate dependence of viscosity for 0.5% gellan gum solutions containing KCl of various concentrations at various temperatures. The *figures* beside each flow curve represent the measurement temperature. **a** Without salt, **b** 10 mM KCl, and **c** 50 mM KCl. Temperature: (○) 60 °C; (▽) 50 °C; (△) 40 °C; (□) 30 °C; (◈) 25 °C; (◇) 20 °C; (⊕) 15 °C; (⊞) 10 °C, (✙) 5 °C



structure where the chains were already conformationally immobile (typical of weak gel behaviour) [31]. Moreover, this system formed an elastic gel during cooling below 30 °C, although the concentration of gellan gum was fairly low, and so the steady-shear viscosity could not be obtained.

Figure 6 shows the shear-rate dependence of viscosity for 0.5% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. From dynamic viscoelastic measurements, $T_{ch}$ were determined to be 26, 30.5 and 34 °C for 0.5% gellan gum solution containing 0.85 mM $CaCl_2$, 1.7 mM $CaCl_2$ and 3.4 mM $CaCl_2$ (data not shown). As shown in Fig. 6, even at lower concentrations, divalent cations had similar effects on the steady-shear viscosity as monovalent cations. The addition of $CaCl_2$ as well as KCl could significantly shift $T_{ch}$ to higher temperatures, and the degree of shear thinning increased with increasing concentration of salt. In the presence of sufficient $CaCl_2$ (as well as KCl), gellan gum formed a weak gel even at a relatively high temperature, and showed significant shear-thinning behaviour showing no Newtonian plateau because the associations of ordered gellan gum molecules became enough to form intermolecular networks.

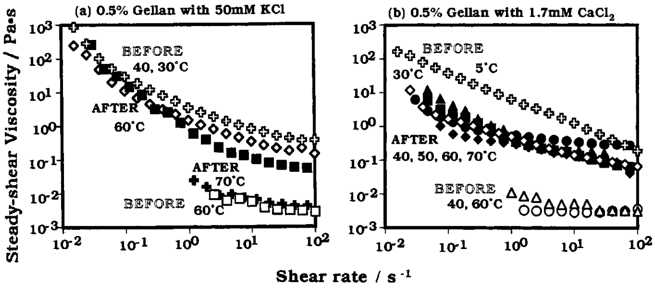


**Fig. 7a, b** Shear-rate dependence of viscosity for 0.5% gellan gum solutions containing 50 mM KCl or 1.7 mM $CaCl_2$ at various temperatures. Each *open symbol* shows the flow curve at each measurement temperature. The *figures* beside each flow curve represent the measurement temperature. Each *closed symbol* shows the flow curve at each measurement temperature after the thermal-scanning process (60–5 °C setting temperature)

## Different effects of monovalent and divalent cations

Figure 7 shows the shear-rate dependence of viscosity for 0.5% gellan gum solutions containing 50 mM KCl or 1.7 mM $CaCl_2$ at various temperatures. These experiments were carried out for a system with sufficient salt where the viscosity did not show the Newtonian plateau even at low shear rates at temperatures below $T_{ch}$. In Fig. 7, each open symbol shows the flow curve at each measurement temperature (before), and each closed symbol shows the flow curve at each measurement temperature after the thermal scanning process in which each system was cooled from 60 to 5 °C at 1.0 °C/min and was then heated up to the measurement temperature at the same scanning rate (after). In the presence of 50 mM KCl (Fig. 7a), at 60 °C, the gellan gum solution had a quasi-Newtonian region up to a fairly high shear rate: however, it changed to shear-thinning behaviour at 40 °C. Moreover, below 30 °C, this system formed an elastic gel, and so the steady-shear viscosity could not be measured. After the cooling and heating process, the gellan gum solution with 50 mM KCl showed shear-thinning behaviour at 60 °C; however, the viscosity was less dependent on shear rate in the heating process up to 70 °C and this solution had almost the same viscosity as that at 60 °C without the thermal-scanning process. In the presence of 1.7 mM $CaCl_2$ (Fig. 7b), the gellan gum solution showed a behaviour close to Newtonian flow at 40 °C, (although gellan gum with 50 mM KCl showed significant shear-thinning behaviour even above 40 °C), and it changed to shear-thinning behaviour below 30 °C. Gellan gum solutions in the presence of 50 mM KCl formed an elastic gel below 30 °C; however, the gellan gum solution in the presence of 1.7 mM $CaCl_2$ could not form an elastic gel even during cooling down to 5 °C. However, after the cooling and heating processes, for the gellan gum solution with 1.7 mM $CaCl_2$, the steady-

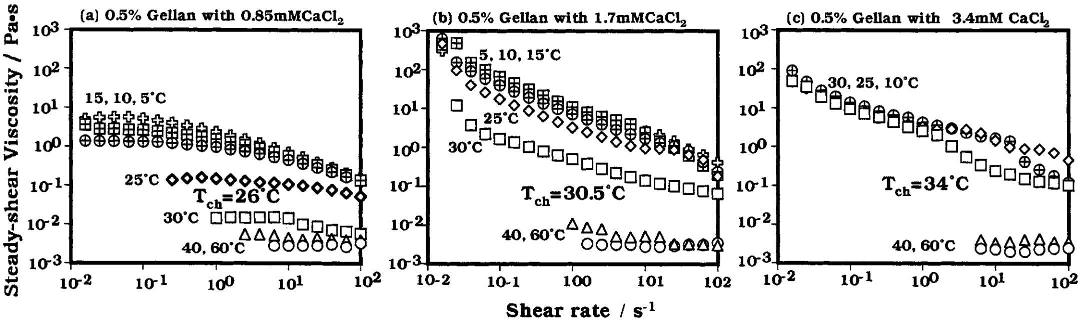


**Fig. 6a–c** Shear-rate dependence of viscosity for 0.5% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. The *figures* beside each flow curve represent the measurement temperature. **a** 0.85 mM $CaCl_2$, **b** 1.7 mM $CaCl_2$, and **c** 3.4 mM $CaCl_2$. Temperature: (○) 60 °C; (△) 40 °C; (□) 30 °C; (◇) 25 °C; (⊕) 15 °C; (⊞) 10 °C, (✥) 5 °C

shear viscosity showed extreme sensitivity to shear rate even on heating up 70 °C and this solution had a significantly larger viscosity than that at 60 or 40 °C without the thermal scanning process.

As illustrated in previous studies [22, 23], the thermal behaviour in the presence of sufficient divalent cations was quite different from that in the presence of sufficient monovalent cations deduced from thermal scanning rheological measurements. During cooling, both moduli for a 1% gellan gum solution in the presence of sufficient divalent cations increased at a lower temperature than in the presence of sufficient monovalent cations; however, in the presence of sufficient divalent cations, no remarkable change in either modulus was observed during heating up to 60 °C.

Therefore, the steady-shear viscosity results as well as the dynamic viscoelastic results suggested that in the presence of divalent cations, the ordered structures of gellan gum formed by cooling became markedly thermally stable, and these ordered structures in the presence of divalent cations were essentially different from those in the presence of monovalent cations.

## References

1. Harris P (ed) (1990) Food gels, Elsevier, London, p 1–476
2. Nishinari K, Doi E (eds) (1994) Food hydrocolloids: structures, properties and functions. Plenum, New York, p 1–510
3. Jansson P, Lindberg B, Sandford PA (1983) Carbohydr Res 124:135–139
4. O'Neill MA, Selvenderan RR, Morris VJ (1983) Carbohydr Res 124:123–133
5. Sanderson GR (1990) In: Harris P (ed) Food gels. Elsevier, London, pp 201–232
6. Morris ER (1994) In: Nishinari K, Doi E (eds) Food hydrocolloids: structures, properties and functions, Plenum New York, p 201–210
7. Robinson G, Ross-Murphy SB, Morris ER (1982) Carbohydr Res 107:17–32
8. Shi X (1990) Thesis. Centre de Recherches sur les Macromolecules Vegetables-CNRS, l'Universite Joseph-Fourier, Grenoble (in French)
9. Manning CE (1992) Thesis. Cranfield Institute of Technology, Silsoe College, Silsoe
10. Moritaka H (1993) Thesis. Showa Women's University, Tokyo (in Japanese with English summary and figure captions)
11. Miyoshi E (1996) Thesis. Faculy of Human Life Science, Osaka City University
12. Sanderson GR, Clark RC, Ortega D (1988) In: Phillips GO, Wedlock DJ, Williams PA Gums and stabilisers for the food industry, vol 4. IRL Oxford, p 301–309, 219–229
13. Duran E, Costell E, Izquierdo L, Duran L (1994) Food Hydrocolloids 8:373–381
14. Nishinari K (1997) Colloid Polym Sci 275:1093–1107
15. Ross-Murphy SB, Morris VJ, Morris ER (1983) Faraday Symp Chem Soc 18:115–129
16. Richardson RK, Ross-Murphy SB (1987) Int J Biol Macromol 9:257–264
17. Rochefort WE, Middleman S (1987) J Rheol 31:337–369
18. Ross-Murphy SB (1994) In Ross-Murphy SB (ed) Physical techniques for the study of food biopolymers. Blackie, London, p 382–385
19. Miyoshi E, Takaya T, Nishinari K (1994) Food Hydrocolloids 8:505–527
20. Miyoshi E, Takaya T, Nishinari K (1994) Food Hydrocolloids 8:529–547
21. Miyoshi E, Takaya T, Nishinari K, (1995) Macromol Symp 99:83–91
22. Miyoshi E, Takaya T, Nishinari K (1995) Thermochim Acta 267:269–287
23. Miyoshi E, Takaya T, Nishinari K (1996) Carbohydr Polym 36:109–120
24. Morris ER, Ross-Murphy SB (1981) In: Techniques in carbohydrate metabolism. B310, 1–46
25. Tanaka Y, Sakurai M, Nakamura K (1996) Food Hydrocolloids 10:133–136
26. Graessley WW (1974) Adv Polym Sci 16:1–179
27. Morris ER (1982) In Phillips GO, Wedlock DJ, Williams PA (eds) Gums and stabilisers for the food industry, vol 2. Pergamon, Oxford, pp 57–78
28. Clark AH, Ross-Murphy SB (1987) Adv Polym Sci 83:57–192
29. te Nijenhuis K (1996) Adv Polym Sci 130:1–267
30. Almdal K, Dyre J, Hvidt S, Kramer O (1993) Polym Gels Networks 1:5–17
31. Doi M, Edward SF (1986) In: The theory of polymer dynamics. Oxford, pp 1–399
32. Richardson RK, Ross-Murphy SB (1987) Int J Biol Macromol 9:250–256
33. Robinson G, Manning CE, Morris ER (1991) In: Dickinson E (ed) Food polymers, gels and colloids. Royal Society of Chemistry, London, pp 22–33
34. Cesaro A, Gamini A, Navarini L (1992) Polymer 33:4001–4008
35. Ogawa E (1996) Carbohydr Polym 30:145–148
36. Okamoto T, Kubota K (1996) Carbohydr Polym 30:149–153
37. Dentini M, Coviello T, Burchard W, Crescenzi V (1988) Macromolecules 21:3312–3320
38. Graessley WW, Segal L (1969) Macromolecules 2:49–57
39. Fouissac E, Milas M, Rinaudo M (1993) Macromolecules 26:6945–6951
40. Mo Y, Takaya T, Nishinari K, Kubota K, Okamoto A (1999) Biopolymers (in press).
41. Crescenzi V, Dentini M, Coviello T, Rizzo R (1986) Carbohydr Res 149: 425–432
42. Kobayashi Y, Okamoto A, Nishinari K (1994) Biorheol 31:235–244