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# Sol-gel Transition of Biopolymer Dispersions

Katsuyoshi Nishinari

Faculty of Human Life Science, Osaka City University, Sumiyoshi, Osaka 558-8585, Japan

**SUMMARY**: Sol-gel transition of dispersions of biopolymers, which are used widely in food, cosmetics, biomedical and related industries, is classified by the temperature dependence of storage shear modulus. Sol-gel transition of gellan gum solution is described well by a criterion of Winter-Chambon. Too fast gelation of dispersions of konjac glucomannan in the presence of excessive amount of alkaline coagulant and/or at higher temperatures leads to a formation of gels with lower modulus. A solution of xyloglucan from which a certain amount of galactose residues is removed forms a gel on heating and reverts into a solution on further heating. The lower temperature transition of this xyloglulcan solution is induced by hydrophobic interaction.

## Introduction

Gelling biopolymers have been used to modify the texture or to control the rheological properties of foods. Rheological properties of solutions of these biopolymers have been studied extensively$^{1,2)}$. Frequency and temperature dependence, and the time evolution of storage and loss shear moduli have been observed together with differential scanning calorimetry (DSC) to understand the gelation mechanism$^{3)}$. The small deformation rheology has the advantage because it is easier to obtain reproducible results and it does not break the structure being formed during gelation.

There are various biopolymers which form a gel on heating such as konjac glucomannan in the presence of alkaline coagulant or soybean glycinin in the presence of glucono-delta-lactone; solutions of both polymers form thermo-irreversible gels. Gelatin, agarose, carrageenans, gellan, and many other polysaccharides form a gel when their solutions are cooled, and these gels are thermo-reversible. Solutions of methylcellulose, xyloglucan from which some galactose residues are removed form a gel on heating, and these gels are also thermo-reversible. Recently, the interaction between different biopolymers has been studied extensively since the discovery of gel formation by mixing non-gelling agents such as xanthan and

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galactomannan derived from locustbean. It has been expected to find other new and useful combinations to develop new gelling agents4).

Fig. 1 shows the temperature dependence of storage modulus for four dispersions. In case (a) the gel is formed on cooling as in agarose, gellan, carrageenan, gelatin etc, and in case (b) the gel is formed on heating as in methylcellulose, methylhydroxylpropylcellulose, ovalbumin, and in case (c) the gel is formed both at lower temperatures and at higher temperatures as in the mixture of gelatin and methylcellulose, and in case (d) the gel is formed only at intermediate temperatures as in xyloglucan from which some galactose residues are removed. Every case is possible, and it suggests the infinite possibility of the science and technology of gels and gelling processes. This paper describes some recent development in understanding the sol-gel transition of three polysaccharides which show different thermal behaviours such as cases (a), (b) and (d) in Fig. 1.

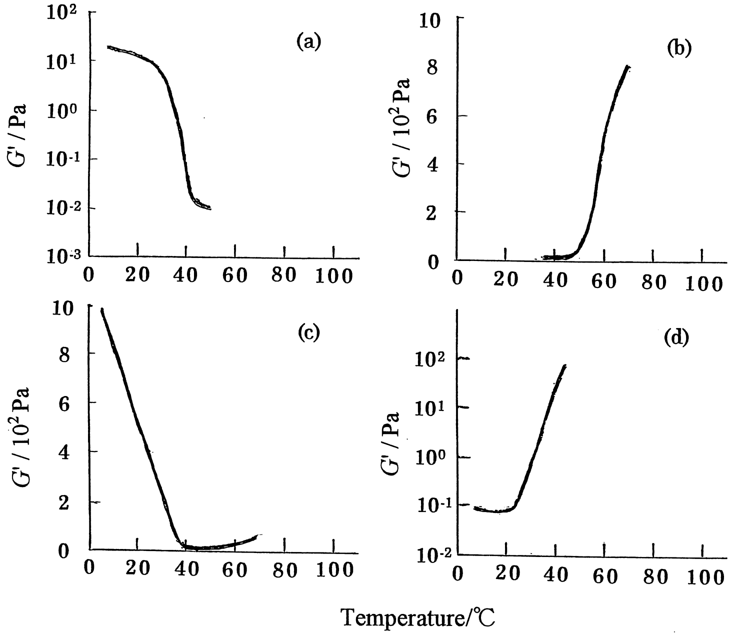

Fig. 1: Temperature dependence of storage shear modulus $G'$ for (a) 3% solution of sodium type gellan5), (b) 3% solution of methylcellulose6), (c) mixed solution of 5% gelatin and 2% methylcellulose7), (d) 1% solution of xyloglucan from which 43% galactose was removed8). Storage shear modulus at higher temperatures >50℃ have not been measured but should decrease with increaseing temperature.

## Sol-gel transition of gellan gum solutions

Gellan gum is a microbial polysaccharide consisting of a tetrasaccharide unit, glucose-glucuronic acid-glucose-rhamnose, and has attracted much attention because it forms a transparent, heat- and acid-resistant gel.

Since even a subtle change in metal ion content or a molecular weight distribution will cause a drastic change in physico-chemical properties of polyelectrolyte solutions such as gellan gum, the common sample of sodium type gellan gum was prepared by San-Ei-gen F.F.I. and distributed to 17 laboratories with different disciplines of the working party on gellan gum organised in the Research Group on Polymer Gels affiliated to the Society of Polymer Science, Japan. This collaborative research began ten years ago using the common sample and the results were published twice9,10). The third common sample used was a sodium type which was easily soluble in cold water, and it was possible to determine the weight average and number average molecular weights to be $M_w$ = 9.5x$10^4$ by light scattering11), and $M_n$= 5.8x$10^4$ by osmotic pressure measurement12) respectively. Results obtained by this collaborative research group by using X-ray small angle scattering, circular dichroism, NMR, ESR, DSC, dielectric and viscoelastic measurements are now in press as a special issue of the Progress in Colloid and Polymer Science. The present section concentrates mainly on rheological and DSC studies of this gellan.

The state diagram of gellan gum aqueous solution is shown in Fig. 2(a)5). The coil-helix transition temperature $T_{ch}$ was determined as the temperature at which loss shear modulus *G*" as a function of temperature showed a steep rise on cooling. This temperature coincided well with the transition temperature at which the molar ellipticity at 202nm changed drastically in the circular dichroism measurements13). The sol-gel transition temperature $T_{sg}$ was determined as the temperature at which storage shear modulus *G*' took over loss shear modulus *G*" on cooling, and *G*' showed a step-like change.

Frequency dependence of storage and loss moduli, *G*' and *G*", for 1~3.5 wt% sodium type gellan gum aqueous solutions at various temperatures is shown in Fig. 2 (b-d)5). A 1% solution shows a typical dilute solution behaviour of flexible linear polymers at 0~30℃, i.e., *G*">*G*' for all the frequencies accessible, and both moduli increase with increasing frequency (Fig. 2(b)). The frequency dependence of both moduli could be well approximated by *G*" ~

$\omega$ and $G' \sim \omega^2$ at low frequencies, which is a characteristic feature for dilute polymer solutions. A 2.5% solution also shows a dilute solution behaviour above $T_{ch}$ = 37.7℃, but $G'$ and $G''$ show a cross-over between $T_{ch}$ = 37.7 and $T_{sg}$ = 26℃; $G''>G'$ at lower frequencies but $G'>G''$ at higher frequencies, a so-called concentrated solution rheological behaviour where the molecular chains disentangle during a long period of oscillation at low frequencies, and the solution behaves as a viscous liquid, whilst the molecular chains do not disentangle during a short period of oscillation at high frequencies, and their entanglement points play a role of temporary knots of three-dimensional network, and as a result the behaviour of the

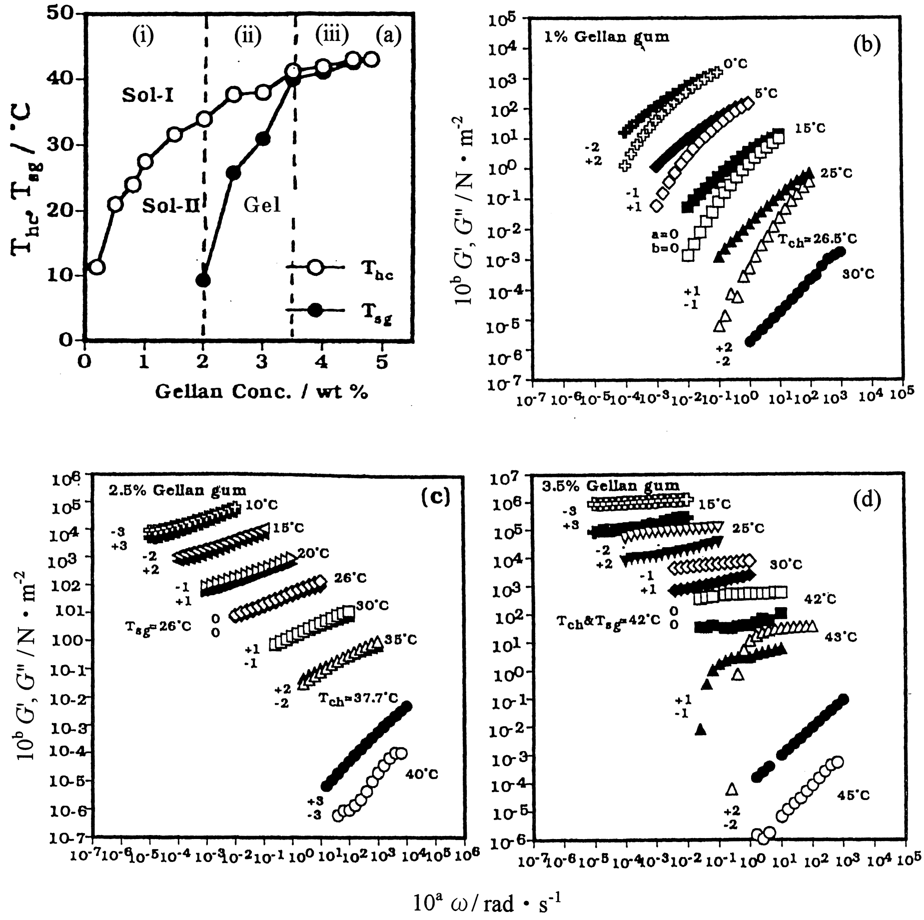

Fig. 2: State diagram (a) and frequency dependence of storage (open) and loss (closed) shear moduli for 1%(b), 2.5%(c), and 3.5%(d) gellan gum solutions. The data are shifted along both the horizontal and vertical axes by shift factors a and b, respectively to avoid the overlapping.

solution tends to that of an elastic solid (Fig.2(c)); at the sol-gel transition temperature $T_{sg}$ = 26℃, the solution is in the critical gel state which Winter-and Chambon proposed, i.e., both moduli showed the same frequency dependence, $G'' \sim G' \sim \omega^{0.5}$ ; below the sol-gel transition temperature the solution tends to show a weak gel behaviour, i.e., $G'$ is slightly larger than $G''$ and both moduli are only slightly dependent on the frequency. A 3.5% solution shows a concentrated solution behaviour at 43℃, however, it turns to an elastic gel behaviour on lowering the temperature only 1℃ (Fig. 2(d)).

It is well known that physico-chemical properties of anionic polysaccharides such as $\kappa$-carrageenan and gellan are strongly influenced by the addition of salts$^{1-3)}$. The endothermic peak in the heating DSC curves and the exothermic peak in the cooling DSC curves shifted to higher temperatures with increasing concentration of the added salt. The difference between the exothermic peak temperature in the cooling DSC curve and the endothermic peak temperature in the heating DSC curve became larger above a certain concentration of the added salt$^{5)}$. The temperature dependence of $G'$ was shown with a cooling DSC curve for 1% gellan gum solution in the presence of CsCl at the same cooling rate (Fig. 3). The lower temperature DSC peak in the presence of 10mM CsCl was not observed in the previous common sample because the main exothermic peak was broader in the previous sample$^{14)}$. The lower temperature DSC peak corresponded well with the sol-gel transition where $G'$ and $G''$ showed a cross-over in the thermal scanning rheology. The minimum content of the salt

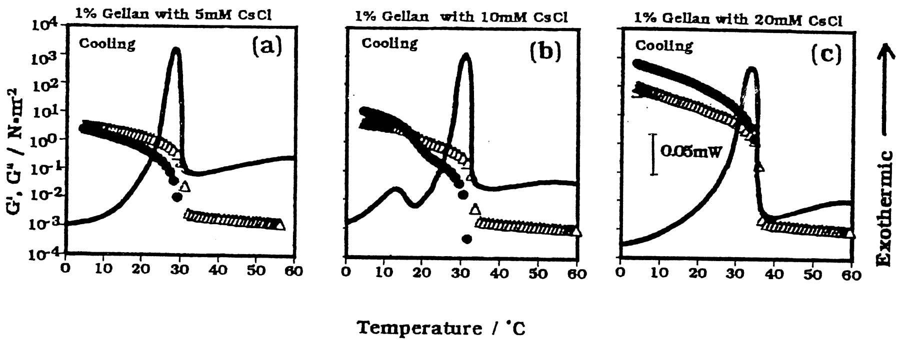

Fig.3: Cooling DSC curves and the temperature dependence of storage shear modulus $G'$(●) and loss shear modulus (△) at 0.1 rad/s observed at the same cooling rate (0.5℃/min) for 1 % gellan gum solutions in the presence of CsCl of various concentrations.

which induces the gelation of 1% gellan gum solution was in the following order: $Li^+ > Na^+ > K^+ > Cs^+$, which also coincides with the dynamic hydration number[5)].

Effects of glucose, fructose, sucrose and trehalose on the gel-sol transition of gellan gum were also examined by rheology and DSC and all these sugars except fructose were found to promote the gel formation of gellan gum[5)].

## Sol-gel transition of konjac glucomannan dispersions

Konjac glucomannan (KGM), composed of mannose and glucose, and the ratio of mannose to glucose is about 1.6:1, has been used as an important ingredient in the traditional Japanese foods. Although a KGM dispersion does not form a gel without adding the alkaline coagulant, mixtures of KGM and other hydrocolloids such as $\kappa$-carrageenan or xanthan form a gel, and have been used as dessert jellies. This synergistic interaction has been studied extensively recently[15)].

The frequency dependences of storage and loss shear moduli for konjac glucomannan solution from 0.35 to 1.4% show concentrated solution behaviours[16)]. The time dependence of the storage and loss shear moduli at a constant temperature and at a constant frequency was observed for konjac glucomannan solutions in the presence of a constant concentration of alkaline coagulant $Na_2CO_3$ for KGM solutions with different molecular weights or with various polymer concentrations[17)]. The increase in the storage modulus was well approximated by a first order kinetic equation $G'(t) = G'_{sat}\,[1 - e^{-k(t-t_0)}]$, where $G'_{sat}$ is the plateau value of $G'$ after a long time, $k$ is the rate constant of gelation, and $t_0$ is the gelation time. The gelation time $t_0$ became shorter, and $k$ and $G'_{sat}$ became larger with increasing molecular weight or concentration. $G'_{sat}$ varied with the concentration as $c^{2.55}$. The time dependence of the storage and loss shear moduli in the presence of a constant alkaline coagulant and at a constant frequency was observed for konjac glucomannan solutions at various temperatures from 50 to 80℃. The gelation time $t_0$ became shorter and the rate constant $k$ and the saturated value of storage shear modulus $G'_{sat}$ became larger with increasing temperature, however, $G'$ seemed to decrease after a certain time at higher temperatures such as 75 and 80℃. Too fast gelation in the presence of excessive alkaline coagulant led to the smaller $G'_{sat}$ although $t_0$ became shorter and the rate constant $k$ became larger (Fig. 4)[17]. This may be attributed to one of the following reasons: 1) Water exudes out when the gelation proceeds too fast and the slippage occurs between the cone plate geometry and the sample dispersion, 2) Very weak ordered structure is formed when the gelation proceeds too fast and this structure is broken during the oscillatory measurement although the amplitude is very small. The similar situations have been observed in the gelation of soybean protein and $\kappa$-carrageenan.

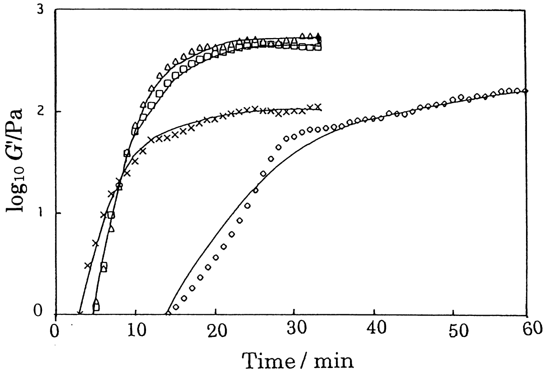

Fig. 4 : The temperature dependence of storage shear modulus of 1% konjac glucomannan dispersion in the presence of alkaline coagulant $Na_2CO_3$ of different concentrations. ◇: 0.5M, □: 1.0M, △: 1.5M, ×: 2.0M. Measurement temperature: 60℃, frequency: 1rad/s. Symbols represent the experimental values and the solid lines represent the calculated curves using the first order kinetic equation[17].

## Sol-gel transition of xyloglucan solutions

Xyloglucan extracted from tamarind seed is a $\beta$-glucan backbone with $\alpha$-D-xylosyl residues attached to the 6-position of $\beta$-glucosyl residues. It was found that when some of the galactose side chains are removed by an enzyme, the solution of xyloglucan formed a gel on heating. Shirakawa et al found that xyloglucan from which galactose residues were removed formed a gel on heating and returned into sol state on further heating, and that this transition was thermally reversible (Fig. 5)[18]. The difference between two sol states at higher temperatures and at low temperatures were not clarified yet, and should be studied in the future.

As has been observed for methylcellulose solutions$^{6)}$, an endothermic peak was observed in the heating DSC curve accompanying the sol to gel transition$^{8)}$. In most other gelling polysaccharides which form a gel on cooling, an exothermic peak has been observed on cooling accompanying the sol-to-gel transition, and an endothermic peak has been observed on heating accompanying the gel-to-sol transition$^{3)}$. In these cold setting gels, the molecular forces which take part in forming junction zones are mainly hydrogen bonds while in the heat setting gels like methylcellulose or the modified xyloglucan, gel is believed to be formed by the hydrophobic interaction. The change in enthalpy accompanying the formation of hydrogen bond is negative, while the enthalpy change accompanying the formation of hydrophobic interaction can be positive or negative. The endothermic enthalpy observed for gelation of methylcellulose (16 J per one gram polymer)$^{19)}$ and methylhydroxylpropylcellulose (6.9J/g)$^{20)}$ is far smaller than that for gelatin (24.2 J/g)$^{21)}$, κ-carrageenan (46 J/g)$^{22)}$, and agarose (40 J/g)$^{23)}$. As is stated above, the latter group forms a gel on cooling by hydrogen bonds, while the former group forms a gel on heating by hydrophobic interaction. It is well known that the energy for hydrophobic interaction is about one-fourth of that for hydrogen bond$^{24)}$. The endothermic enthalpy observed for gelation of xyloglucan was 4.4 J/g$^{8)}$, and is the same order of the magnitude for the former group, suggesting that the gel of xyloglucan is formed by hydrophobic interaction.

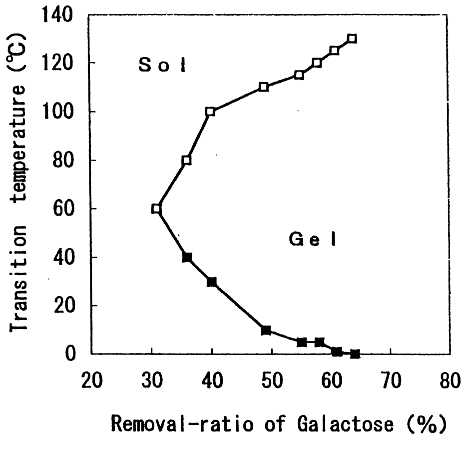

Fig.5: The sol-gel transition temperature diagram of 2% xyloglucan solution as a function of the removal ratio of galactose from xyloglucan. □: high-temperature transition point, ■: low-temperature transition point$^{18)}$.

## Conclusion

It has been shown that helix formation is pre-requisite for gel formation in gellan gum solution, and that gel is not formed below a certain critical concentration, and that above a certain concentration gel is formed concurrently with helix formation. This is in agreement with the classical picture proposed earlier for sol-gel transition of $\iota$ -carrageenan[25]. However, another picture for gels, a "fibrous model" is proposed recently based on atomic force microscopic obsevation[26]. It is necessary to study further using the common sample with different molecular weights with narrow molecular weight distribution. It has been shown that gel formation of konjac glucomannan dispersions on heating in the presence of alkaline coagulant proceeds faster with increasing temperature, concentration of KGM/alkaline coagulant, and molecular weight of KGM. However, too fast gelation leads to a gel with a smaller elastic modulus. This should also be clarified by further study. It has been suggested that gel formation of solutions of xyloglucan from which galactose residues are removed is induced by hydrophobic interaction. The higher temperature gel-sol transition should be studied in the future. Further study on the relation between chemical structure of biopolymers and sol-gel transition is required to develop further utilisation of these biopolymers.

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