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Rheological and Thermal Studies on the Sol-Gel Transition of Gellan Gum and Mixed Polysaccharides

A Thesis for Doctor of Philosophy

1996

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Contents

INTRODUCTION

1. Role of Gelling Agents in Foods

For hundreds of years, Japanese people have been enjoying traditional gel-like foods such as 'tofu', 'konnyaku' and 'kamaboko'. Recently, other foods with unique textures, such as 'natadecoco', 'konjac jellies' and 'gum-like candy', have attracted much attention and have been enormously popular.

Japanese people are very conscious of food textures. So, the development of food products should be aimed at achieving desirable physical structures and perceived eating quality. Since Japanese people prefer to enjoy the natural flavour of food, the structural and textural properties of food materials are especially important factors by which to judge the quality of foods. This tendency is seen not only in Japan but also in the western world. Eating should not be only to satisfy our appetites but also to give pleasure. We are exposed to a great range of foods and can make our choice depending on individual preference.

Thus, the sensory quality of food is as important a functional property as nutritional value and physiological function. This quality is a psycho-physical phenomenon, and has been systematized in accordance with the senses by which the various attributes of quality are perceived by the consumer; appearance and colour as sensed by the eyes, flavour as sensed by the papillae on the tongue, aroma as sensed by the olfactory epithelium of the nose, and texture as sensed by the nerve endings that subserve muscle (Szczesniak and Kleyn, 1963). However, there is no clear boundary between these quality attributes, none is entirely independent. They overlap and each is influenced by other attributes (Kramer, 1968).

Texture is the main component of sensory quality. It covers all kinesthesis (the muscle sense) and haptaesthesis (the skin sense) of foods, which mainly includes touch or feel in mouth and skin (Sherman, 1969). Texture is related to the structure of food, such as the arrangement of constituents, the dispersion of particles, and the alignment of fibres. Thus, texture is mainly related to the rheological properties of foods, whereas the other sensory qualities, taste, aroma and flavour, are mainly controlled by the chemical composition (Nishinari, 1991). There are two approaches to evaluate eating quality of food materials related to texture (Muller, 1973). One is the direct sensory approach which deals with perception of textural properties of foods through the human sensory response. We touch the food with the hand, break it up and bite it with the teeth, chew and swallow it and brain integrates these sensations into a total impression of quality. Experimentally, the texture of foods is evaluated by a group of people trained to evaluate the key characteristics of foods and the results are treated statistically. Another approach is the physical measurement of mechanical properties which are independent of human beings. This method uses instruments, and has been called 'objective'. Rheology is a branch of physics which deals with the deformation and flow of material, both solid and fluid (Reiner, 1960), and one of the main purposes of food rheology is to develop instrumental methods which facilitate prediction of consumers' evaluation of the textural properties of foods (Scott Blair, 1958). Thus, the instrumental (objective) tests for texture concern the deformation and flow behaviour of food material, and these results should be expressed in terms of well defined physical parameters (Sherman, 1982). The interaction between these haptaesthetic and rheological approaches related to texture of foods belongs to 'psychorheology' which is the study of the relationship between subjective evaluation and rheological measurement. Its ultimate goal is to establish mathematical equations or relations that permit the scientist to predict the sensory characteristics of food materials from physical measurements. However, instruments cannot appreciate texture like a human being. Therefore, psychorheology tries to describe the relevant physical characteristics of food materials and to analyse subjective-objective correlations (Moskowitz et al., 1973).

The relation between the perceived "thickness" of fluid foods and beverages in the mouth, and the viscosity by instrumental evaluation has been studied by many researchers. Wood (1968) has concluded that the thickness is perceived in the mouth at a constant shear rate of about 50 s^{-1}. Since viscosity η is related to shear rate $\dot{\gamma}$ and shear stress τ, Shama and Sherman (1973) investigated this problem using a wide range of foods with different flow properties. They have concluded that the effective oral shear rate decreases and the shear stress increases with increasing viscosity, hence, thinner fluid foods are moved around in the mouth more rapidly than thicker ones. E.R.Morris et al. (1983) suggested that for Newtonian solutions such as sugar syrups, the relationship between objective stimulus (viscosity η) and subjective response (thickness T) follows a 'power law' ($T = 1.69 \cdot \eta^{0.22}$) analogous to Stevens' psychophysical law (Stevens, 1960). Baines and Morris (1988) have found that the rotational viscosity at a fixed shear rate of $50 s^{-1}$ correlates well with 'thickness', 'stickiness' and 'sliminess' for 'true' solutions, but, seriously underestimates the perceived texture of 'weak gels' (as described in section 2 "Definition of the Term Gel" of Introduction), where the tenuous network structure is disrupted during the large deformation measurements. However, they have also found that non-destructive oscillatory measurements of η^* at 50 $rad \cdot s^{-1}$ correlate directly with perceived thickness, stickiness and sliminess in both true solutions and weak gels. This result indicates that oral evaluation of the texture of 'weak gel' is based predominantly on the viscoelastic properties of the intact network structure, rather than on those of isolated species released after rupture of the network by shear (Baines and E.R.Morris,1988 and E.R.Morris, 1994a). Morris et al. investigated the effects of texture on flavour/taste intensity, since it is well known in the food industry that the concentrations of compounds providing flavour or taste attributes (sweet, sour, bitter and salty) required to produce the same subjective intensity are often much higher in thickened products than in more fluid systems. It is well known in Japan that 'mizuyokan' (agar jelly dessert), one of the Japanese traditional foods, contains 30% sugars, however, if fluid foods or beverages contain the same amount of sugars they are too sweet to drink (Lowe, 1959). Baines and E.R.Morris (1988) found that weak gels such as xanthan gum, which show extreme shear-thinning, give almost perfect flavour release. They suggested that, although the stress and strain at which "true" gels break correlate with, respectively, perceived "hardness" and "brittleness", flavour release from these gels is independent of "hardness", but correlates directly with "brittleness". Brittle gels such as gellan gum give better release than more elastic materials (E.R.Morris, 1994a). These investigations showed that the textural attributes perceived by human beings could be expressed in terms of well defined physical parameters.

Recently, some lucid and enjoyable textbooks on eating quality of food products have been published in Japan (Matsumoto, 1991 and 1995; Yamano and Yamaguchi, 1994).

With increasing use of processed and simulated foods, the consumer wants new eating quality related to texture rather than taste, such as salty, sweet, sour or bitter. Thus, product developers are making great efforts to create new textured food products and the need for better methods of measuring and controlling texture is growing. These physical properties of processed and simulated foods are mainly controlled by hydrocolloids such as polysaccharides or protein (Blanshard and Mitchell, 1988; Glicksman, 1986, Nishinari. 1988). Many food hydrocolloids have always played a significant role in the field of food science and technology and have been long used as texture modifiers, emulsion stabilisers, water absorption agents, gelling and thickening agents, and many other desirable characteristics (Nishinari and Yano, 1990; Nishinari and Doi, 1994). All these functions of food hydrocolloids affect the textural and rheological properties of a product, both immediately after manufacture and during long term storage prior to use. Consequently they can have a strong influence on the acceptability of the product to the consumer (Sherman, 1982). In Japan we have long history of eating gel-like foods such as 'kamaboko'(fish paste), 'tofu'(soybean curd) 'kuzukiri'(kuzu starch gel), 'mizuyoukan' (red bean-agar jelly dessert). These food hydrocolloids are very important for a wide range of food application (Nishinari, 1988).

Generally, food gels can be formed by either a protein or a polysaccharide, however, the resultant gels have different characteristics (Harris, 1990). Polysaccharide gels are characterised by a lower polymer concentration and can be formed by heating and cooling, pH adjustment or specific ion addition. Many polysaccharide gels are thermoreversible. Protein gels are characterised by a higher polymer concentration and are formed almost exclusively by heating except some protein gels formed in the presence of trans glutaminase and gels formed in the presence of rennet (Harris, 1990). Gelatin is one of the most important gelling agents and is a protein of animal origin (Johnston-Banks, 1990). Gelatin forms thermoreversible gels on cooling a suitably concentrated solution (Johnston-Banks, 1990).

Most industrial polysaccharides originate from seaweeds, cultivated plants, or microbial sources (Nishinari and Yano, 1990; Harris, 1990). Gel formation by these polysaccharides is generally associated with the transformation of essentially disordered biopolymers such as random coils to a partly ordered state such as helix or rigid rod. The most important marine polysaccharides are derived from the extracellular matrix of marine algae or seaweeds, which include agar and agarose (Matsuhashi, 1990), carrageenan (Stanley, 1990) and alginates (Sime, 1990). Polymers of the agar and carrageenan types form thermoreversible gels, and in contrast, alginate gels are heat stable to temperatures of >100℃. Pectin and starch gels are the most important gel-forming polysaccharides from plants (cellulose is so insoluble that it gels only after chemical modification). Pectin with a low degree of esterification behaves like alginates and forms a gel in the presence of divalent cations (Rolin and De Vries, 1990). Starch gels may be distinguished from other polysaccharide gels, because on heating, starch granules swell and rupture (at temperatures>60℃), and then at relatively higher concentrations, thermoirreversible gels were formed (Whistler, BeMiller and Paschall, 1984).

Recently, bacteria have become an increasingly important source of polysaccharides, so that the new microbial polysaccharides are of growing commercial importance and are produced on a large scale by industrial fermentation. The most widely used among these materials are xanthan gum and gellan gum. Xanthan gum was first launched as a commercial polysaccharide by Kelco. Inc., and is one of the most intensively studied food polysaccharides. Gellan gum is another superior gelling polysaccharide. It may offer a solution to many of the problems that exist with current gelling agents (Sanderson, 1990), as is described later. More than 900 other gum-forming bacterial polysaccharides have already been isolated. Although all of these bacteria are of scientific interest, however, most are not of commercial value (Sanderson, 1990).

In the past, the nutritional properties of these polysaccharides attracted relatively little attention because they appeared to be of little nutritional value (except for starch). However, many nutritional scientists are now greatly interested in the physiological effects of such polysaccharides as dietary fiber. Moreover, since health authorities in most industrialised countries recommend a reduction of total and saturated fat, there is much interest in the potential of these polysaccharides for the formulation of low-fat and low-calorie foods.

Gellan gum, an anionic polysaccharide produced by *Pseudomonas elodea*, has a complex tetrasaccharide repeating sequence of →3) β-D-glucose,(1→4) β-D-glucuronic acid, (1→4) β-D-glucose, (1→4) α-L-rhamnose (1→, and it has a carboxyl side group (Jansson et al., 1983; O'Neill, et al., 1983), (Figure 1-1). Gellan gum, first discovered as recently as 1978, is being used increasingly in a wide variety of food products in many parts of the world (Sanderson, 1994). The native gellan gum which contains one glycerate substituent per repeating unit and one acetate approximately every second repeating unit, is currently not available. However, gellan gum for microbiological media and related applications (Gelrite) and food-grade gellan gum (Kelcogel) which are low acyl products, are now commercially available (Sanderson, 1990). Native gellan forms soft elastic gels, however, progressive deesterification results in increasingly hard brittle gels (E.R.Morris., 1990a). Extensive safety studies have been conducted to secure the approval for use in foods (Table 1-1), and results from these studies have not given any cause for concern (Sanderson, 1990). Gellan gum is widely used in food industry and biotechnology because it forms a transparent gel with outstanding flavour release, which is resistant to heat and its gel strength is less dependent on pH than many other polysaccharide gels (Sanderson, 1990).

Since agar, carrageenan, alginate, and pectin with a low degree of esterification are simple to use, for a long time, they have been widely used for food products such as dessert jellies. However, agar gels which are formed on cooling become relatively turbid and the gel strength becomes weaker on addition of acid. Pectin with a low degree of esterification can only form gels in the presence of divalent cations. Seaweed which is the source of alginate, carrageenan, agar, is subject to the uncertainties of nature and is not always available in sufficient quantities to satisfy market demand. Moreover, for these polysaccharides, the differences between the commercially available various grades can lead to problems. The reason why gellan gum can solve many problems that exist with these polysaccharides is described as follows. Since gellan gum is a fermentation product, it can be produced on demand and with consistent quality, thus, availability and variability are not concerns (Sanderson, 1990). Gellan gum can function at relatively lower concentrations and be adapted to many applications. This provides effective savings in production costs. Since gellan gum can provide a wide-range of gel textures by careful control of added salts, these gels can give the same texture as other polysaccharide gels or create new textures.

The present research is mainly concerned with the purified sodium form of gellan gum (which is the common sample as described in section 1 "Functional Properties of Gellan Gum" of Chapter I). The aim was to investigate the rheological and thermal properties of gellan gum solutions, by dynamic viscoelastic measurement and differential scanning calorimetry (DSC). In order to clarify the helix-coil and the sol-gel transitions of gellan gum, the rheological and thermal properties of gellan gum solutions with and without salts (sodium chloride, potassium chloride, calcium chloride and magnesium chloride) were investigated and are described in Chapters I& II. The relation between thermal properties and rheological properties, and comparison with results obtained by our coworkers are also discussed in these chapters.

In Chapter III, the rheological and thermal properties of gellan gum solutions with and without sugars and konjac glucomannan of relatively lower molecular weights are investigated, in order to investigate the role of water or hydration in gel structure. In addition, since soft food gels such as dessert jellies are formed by the polysaccharides and very often include sugars, the effects of sugars on the sol-gel transition in gellan gum are described for food application in this chapter.

It is well known that when more than two polymers are mixed, the properties of mixtures are superior to those of either component alone, or change to qualitatively different properties (V.J.Morris, 1986; E.R.Morris, 1990b), which is described as 'synergism'. However, the gelation mechanism of many such mixed gels is unknown, in particular, the synergism of mannan and helix-forming polysaccharides such as carrageenan or agar is a topic of considerable current debate. Thus, in Chapter IV, the synergistic interaction between gellan gum and konjac glucomannans with different molecular weights is described. Also, in Chapter V, the effects of monovalent and divalent cations on the interaction between gellan gum and konjac glucomannan are described. A brief overview of "mixed polysaccharide gels" is described in section 1 of Chapter V.

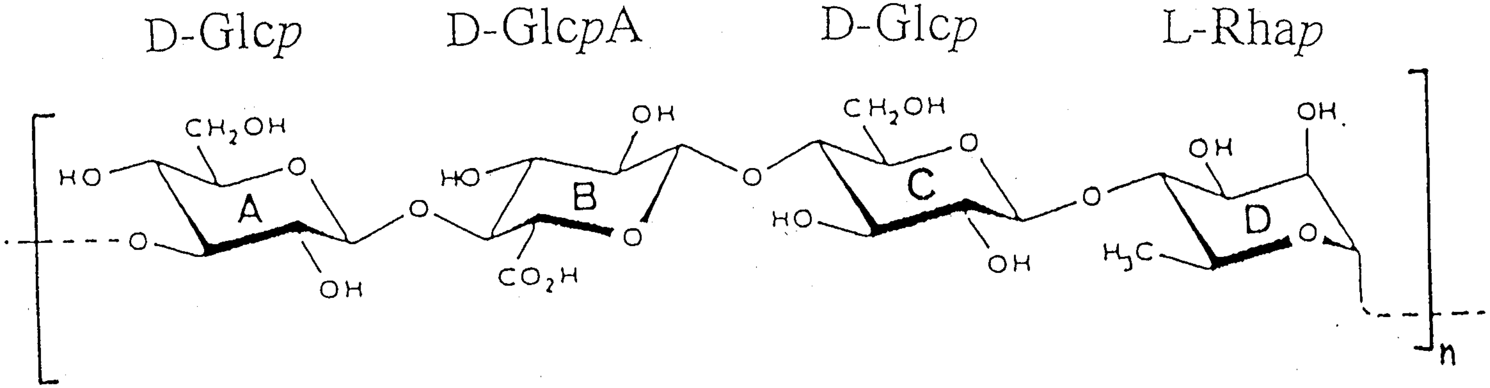

Fig.1-1 Repeat-unit structure of gellan gum consisting of tetrasaccharide.

Table 1-1

Gellan Gum Safety Studies

Animal	Test	Level of safety
Rat	Oral LD_{50}	>5000mg/kg
	4-hour dust inhalation, LC_{50}	>6mg/Litre nominal
	3-month dietary	No sings of toxicity at up 6% of diet
	2-generation reproduction	No adverse effects noted at up 6% of diet
	Teratology	No dose-related effects noted at up to 5% of diet
	In utero/chronic toxicity/ carcinogenicity	
	F_0 generation, 63 days	Possible effect on rate of weight gain of males: no significant other findings. Dietary up to 5% of diet
	F_1 generation, 104 weeks	Treatment produced no over signs of toxicity and no effect on spontaneous tumor profile. Achieved intake ~3g/kg/day in high dose group
Mouse	Chronic dietary, 96-98 weeks	No adverse reaction to treatment noted. Produced no adverse signs of toxicity and no effect on the spontaneous tumor profile. Fed up to 3% of diet, with achieved intake being ~5g/kg/day in high dose group
Dog	Chronic dietary, 52 weeks	No toxicological effect noted after 52 weeks of feeding at concentrations of 3%, 4.5% or 5% (representing daily intakes of ~1.0, 1.5 or 2.0g/kg/day) for both males and females
Monkey	28-day oral	No clinical signs or changes in blood chemistry noted at doses up to 3g/kg
Rabbit	Skin/eye irritation	No skin irritation noted. No eye irritation noted
In vitro	Ames	No negative effect
	USD	No negative effect
	V-79 cell mutagenesis	No negative effect
	Clinical organisms	No significant differences in growth on plates gelled with gellan gum and plates gelled with agar
Humans	23-day	No adverse effects on plasma biochemistry, haematology or urinalysis. Dosed at 200mg/kg/day

(Sanderson, 1990)

2. "Definition of the Term 'Gel' "

Many scientists have made great effort to classify 'gel' systems based on easily recognizable physical and rheological properties (Te Nihenhuis, 1995). However, different types of gels have different structures and are studied by scientists with different backgrounds, i.e. physicists, chemists, chemical engineers, biologists, rheologists, etc., so that it might be difficult to describe a simple definition of a gel. Indeed, as D.Jordan-Lloyd stated in the introduction of 'The problem of gel structure' in 1926, "the colloidal condition, the gel, is one which is easier to recognize than to define, and even recognition is confused by the fact that the limits between gel and sol, on the one hand, and gel and what may be termed curd, on the other, are not precise, but consist of a gradual change." Since this statement was made, scientific understanding has made significant advances and today there are many reviews, including in particular Hermans (1949), Flory (1974), Graessley (1974), Sherman (1979), Ferry (1980), E.R.Morris (1983), Clark and Ross-Murphy (1987), Burchard and Ross-Murphy (1990), Te Nijenhuis (1995).

Flory (1974) has proposed a classification of gels divided into four types: (1) well-ordered lamellar structures, including mesophases; (2) covalent polymer networks, completely disordered; (3) networks formed through physical aggregation, predominantly disordered, but with regions of local order; (4) particulate, disordered structures. According to this definition, undiluted cross-linked rubbers, which consist of one component, are also treated as gels. Hermans (1949) has defined gels as coherent colloid disperse systems of at least two components, and he added that gels exhibit mechanical properties characteristic of the solid state, and both the dispersed component and the dispersion medium extend themselves continuously throughout the whole systems. However, Hermans' definition admits the undiluted materials into the family of gels. Recently, Almdal et al. (1993) have suggested that the term 'gel' is used so indiscriminately that it has become ambiguous, and have proposed some phenomenological definition by focusing on the specific phenomenological characteristics; a gel is a soft, solid or solid-like material of two or more components, one of which is a liquid, present in substantial quantity. Hence, they stated that xylogels and undiluted systems should not be admitted as gels.

We regard a hydrocolloid gel as a material capable of supporting its own weight against gravity (i.e. maintaining its shape) over a practical timescale of days or weeks (E.R.Morris, 1983). Most hydrocolloid gels consist of molecular networks maintained by physical junction zones which can be disrupted by changes in temperature, pH, cation species, or consist of the temporary cross-linking which was formed simply by local chain entanglements resulting from topological constraints (E.R.Morris, 1983, Clark and Ross- Murphy, 1987; Clark, 1992; Ross-Murphy, 1993). These gels belong to the third type of gels in Flory's classification (1974) mentioned above. There are some physical gels which contain some junction zones formed by covalent cross-linking, however, few polysaccharide gels include junction zones formed by covalent bonds.

The theoretical study of synthetic polymer by "tube" or reptation models (Doi and Edwards, 1986) has described successfully the difference between the viscoelastic behaviour of covalently cross-linked gels and the entanglement systems.

Generally speaking, the true gels with intermolecular junctions have a high binding energy and are stable over a long time scale (days or weeks). Entanglement systems behave as liquid at low frequencies, however their behaviour approaches that of a solid at higher frequencies. Hence, this characteristic behaviour reflects the timescale of molecular entanglement (E.R.Morris, 1983; Clark and Ross-Murphy, 1987, Clark, 1992). In other words, at lower frequencies, the molecular chains can disentangle and rearrange during the long period of oscillation, so that the solutions behave like a dilute polymer solution, while at higher frequencies, the interchain entanglements do not have sufficient time to come apart within the period of one oscillation so that the solutions behave like a cross-linked gel. Many systems of practical importance for food applications (e.g. xanthan gum) may be classified rheologically as a weak gel (Clark and Ross-Murphy, 1987, Baines and E.R.Morris 1988, Clark,1992). This falls between two extreme types (I) true gels in which the intermolecular junctions have a high binding energy and (II) concentrated random coil solutions where the interchain 'entanglements' are topological in nature. It seems likely that the weak-gel properties arise from side-by-side association of conformationally ordered chain sequences into junction zones analogous to those in elastic gels, but of lower binding energy (E.R.Morris, 1983). To explain the differences between the strong gels, weak gels and entanglement network systems, it is useful to observe the mechanical spectra of these systems (E.R.Morris, 1983, Clark and Ross-Murphy, 1987, Clark, 1992, Ross-Murphy and Shatwell, 1993). Figure 2-1 (a)~(d) shows the mechanical spectra for a typical dilute polymer solution (a), a concentrated polymer solution (b), a weak gel (c) and a strong gel (d). In the spectrum of a dilute polymer solution (Figure 2-1(a)), the loss shear modulus (G") is substantially larger than the storage shear modulus (G'), and both moduli are strongly frequency dependent (E.R.Morris, 1983, Te Nijenhuis, 1990). The dynamic viscosity ($\eta^*=G^*/\omega$) for a dilute solution shows little change with frequency (i.e. essentially Newtonian behaviour throughout the accessible frequency range) (E.R.Morris, 1983). At lower frequencies, the spectrum of a concentrated polymer solution (Figure 2-1(b)) is closely similar to that of a dilute polymer solution as shown in Figure 2-1(a). At higher frequencies, by contrast, their behaviour approached that shown in Figure 2-1(c) and (d) for polymer gels (E.R.Morris, 1983, Clark and Ross-Murphy, 1987, Te Nijenhuis, 1990, Clark, 1992). η^* for a concentrated polymer solution shows the opposite behaviour; at lower frequencies, η^* is essentially frequency independent, however, the slope of η^* increases with increasing frequency (E.R.Morris, 1983). In the spectrum of a weak gel (Figure 2-1(c)), G' is larger than G" with little frequency dependence throughout the accessible frequency range, and both moduli become more frequency dependent at higher frequencies due to a greater contribution from dynamic entanglements. In the spectrum of a strong gel (Figure 2-1(d)), G' is much larger than G" throughout the accessible frequency range and the absolute values of both moduli were much larger than for weak gels (E.R.Morris, 1983, Clark and Ross-Murphy, 1987, Te Nijenhuis, 1990, Clark, 1992, Ross-Murphy, 1993). G' for a strong gel is essentially independent of frequency, however, G" sometimes shows a shallow minimum at a certain frequency. These behaviours were observed in agar gels (Nishinari, 1976), bovine serum alubumin gels (Richardson and Ross-Murphy, 1981ab), actin gels (Zaner and Stossel, 1983), glycinin gels coagulated in the presence of glucono-δ-lacton (Kohyama and Nishinari, 1992), and heated β-conglycinin gels (Nagano et al., 1994).

Marvin and Oser (1962), Chompff and Prins (1968) and Graessley (1971) have proposed theoretical treatments to explain the dip of G'' in the frequency spectrum. Bell et al.(1984) suggested that an appreciable reduction in G'' corresponds to a slow dissipative process occurring on a time scale of at least several minutes, and reflects the ability of gels to flow and spread, given sufficient time for network rearrangement. However, both weak and strong gels give essentially the same mechanical spectrum, with $G' > G''$, and with both moduli largely independent of frequency, and η^* for both gels decreasing markedly with increasing frequency. Clark and Ross-Murphy (1987) have suggested that it is easy to define the difference between the polysaccharide strong and weak gels by the strain dependence. Figure 2-2 shows the strain dependence of shear modulus (in terms of the reduced modulus G/G_0) of entanglement system, weak gel and strong gel. It is clear that a weak gel is much more strain dependent than the strong gel or the entanglement network. It has been reported (Ross-Murphy, 1984) that the strain dependence of G' was very similar for entanglement systems and strong gels, with the linear viscoelastic strain extending to approximately 25%. For weak gels, the linear viscoelastic strain is usually < 5%. Therefore, it is possible to distinguish between strong gels and weak gels, because they should give rather similar mechanical spectra, but have quite different strain dependence. Moreover, Ross-Murphy and Shatwell (1993) have suggested that the differences between strong gels and weak gels are exhibited by large deformation measurement; at large deformation, strong gels rupture and fail and never recover without melting, and in contrast, weak gels recover and can flow without fracture.

There are number of other methods for determining the gel point, based on determining by the storage modulus G' at a certain frequency as a function of time or temperature. One method is to determine the instant or temperature at which G' becomes to be detected. Another method is to determine the cross-over point of G' and G'' as a function of time or temperature. However, Ross-Murphy (1992) has investigated the gel point of gelatin aqueous solutions by both methods, and concluded that both were rather imprecise. Winter and Chambon (1986) have found experimentally that the mechanical behaviour of crosslinking polymers could be described at the gel point by a power law relaxation shear modulus.

Figure 2-3(a)~(c) shows plots of the frequency dependence of G' and G'' in a pre-gel (state below the gel point), a critical gel (state at the gel point) and a post-gel (state above the gel point). For the pre-gel state (Figure 2-3(a)), a power law is observed at higher frequencies, while at lower frequencies, the typical liquid behaviour is present; $G'(\omega) \sim \omega^2$ and $G'' \sim \omega$. For the critical gel state, both moduli follow a power law throughout the entire frequency range. Beyond gel point, typical solid behaviour is observed at lower frequencies; $G' = G_{\infty}$=constant and $G'' \sim \omega$, however, at higher frequencies, a power law is still observed. Although this method has been developed for covalently cross-linked systems, Te Nijenhuis and Winter (1989) have applied it to physical gels, and since then many investigations have been carried out to determine the gel point for physically crosslinking systems according to the Winter-Chambon method (Te Nijenhuis, 1995). Te Nijenhuis has emphasized that since this experimental model was developed for covalently crosslinked systems, for which they are able to prove by dissolution experiments, that the gel point certainly was attended with power law behaviour of the dynamic moduli, this is not possible for physically crosslinking systems, because physical gels are destroyed by dissolution experiments. However, Te Nijenhuis further states that during the crosslinking process of physically crosslinked systems, a situation arises that resembles closely the critical state of covalently crosslinked systems.

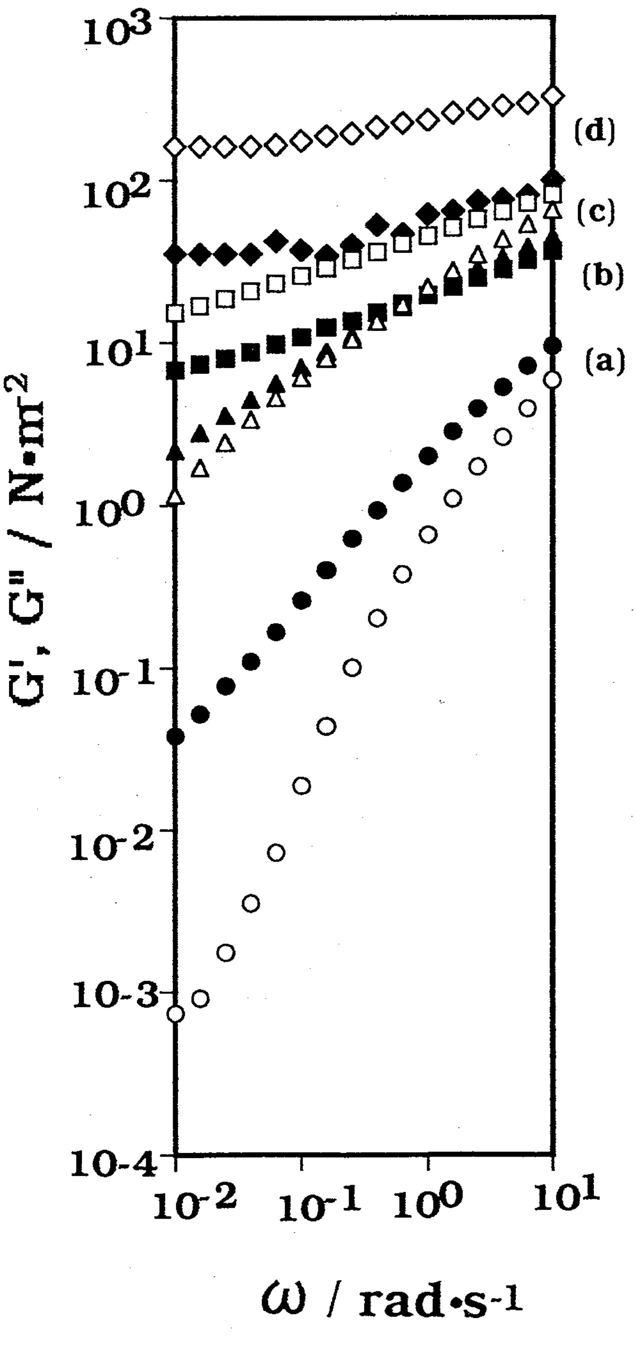

Fig. 2-1 Typical mechanical spectra for 2% gellan gum solutions showing the frequency dependence of G'(○,△,□,◇) and G"(●,▲,■,◆) for
(a) a dilute polymer solution(○,●: 2% gellan alone at 30℃);
(b) a concentrated polymer solution (△,▲: 2% gellan alone at 15℃);
(c) a weak gel (□,■: 2% gellan alone at 0℃);
(d) a true gel (◇,◆: 2% gellan with 1.7mM $CaCl_2$ at 0℃).

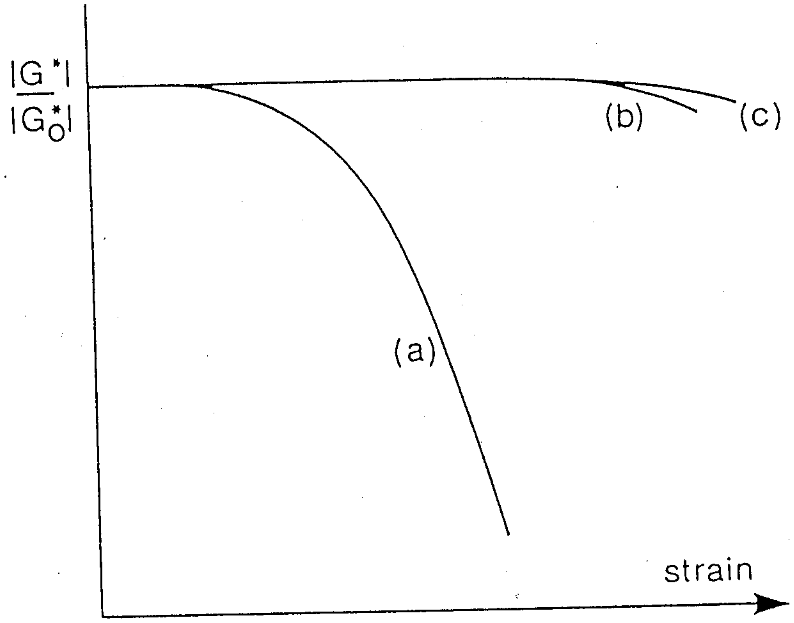

Fig. 2-2 Typical strain dependence of shear modulus (in terms of the reduced modulus G/G_0) for situations of (a) a weak gel, (b) a strong gel, (c) an entanglement network. (Clark and Ross-Murphy, 1987)

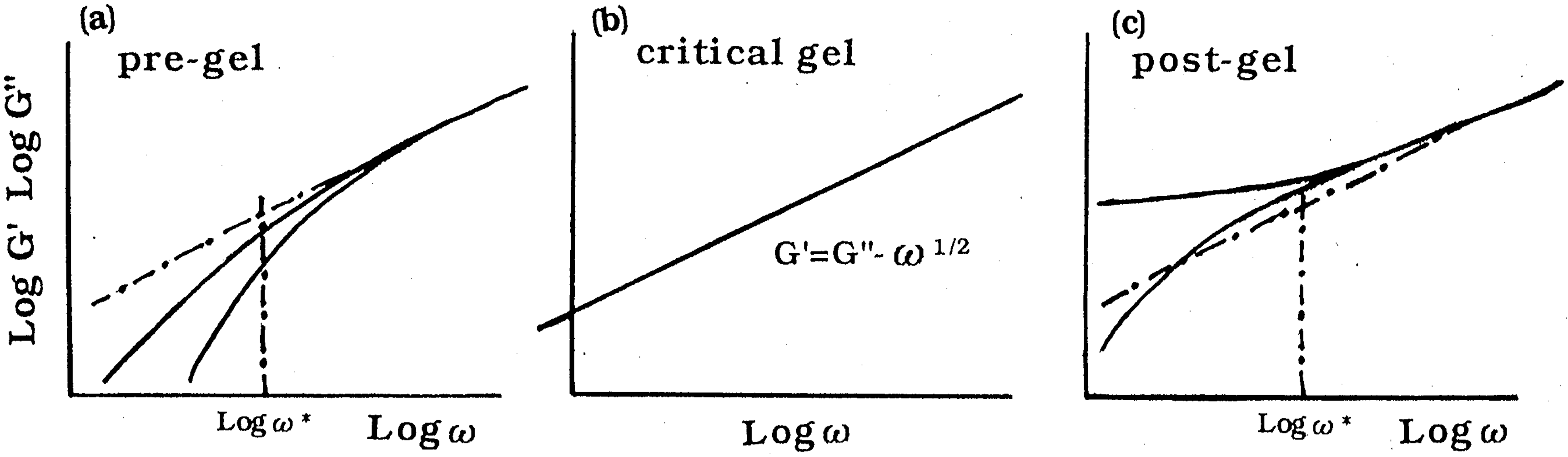

Fig. 2-3 Plots of the frequency dependence of storage modulus G' and G" in a pre-gel (state below the gel point), a critical gel (state at the gel point) and a post-gel (state above the gel point).
(Winter and Chambon, 1986)

I-1. Functional Properties of Gellan Gum

Many research groups have already reported on the physicochemical properties of gellan gum (Jansson et al., 1983 and 1986; O' Neill et al., 1983; Sanderson et al., 1982, 1988 and 1990; Attwool et al., 1985; Attwool and Atkins. 1986; Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986 and 1987; Dentini et al., 1988; Talashek and Brant. 1987; Chapman et al., 1990; Milas et al., 1990; V.J.Morris, 1990; Robinson et al., 1988 and 1991; Tako. 1989; Manning, 1992; Shi, 1990; Owen, et al., 1990; Moritaka et al., 1991 and 1992; Baird et al., 1992; Nakamura et al., 1993; Ogawa, 1993; Okamoto et al., 1993; Shimazaki and Ogino, 1993; Tanaka et al., 1993; Tsutsumi et al., 1993; Yoshida and Takahashi, 1993; Yuguchi et al, 1993; Tang et al., 1994).

Milas et al. (1990) investigated the conformational transition of gellan gum using various techniques (optical rotation, light scattering, viscosity, electrical conductivity). They suggested the conformational transition to be a two-coil-one-double-helix transition. Chandrasekaran et al. (1988, 1990) confirmed the existence of a double-helix structure from X-ray crystallographic studies of polycrystalline and well-oriented samples of the lithium, potassium and calcium salts of gellan gum. In the solid state, the polysaccharide adopts a double helix structure with two left-handed, three-fold chains, each of which is translated by half a pitch (p=5.63nm) with respect to the other. Crescenzi et al. (1987) reported that tetramethylammonium chloride (TMACl) increased the intrinsic viscosity $[\eta]$ of gellan gum solutions at 25°C while the same salt decreased $[\eta]$ at 40°C, and have suggested that at lower temperatures gellan gum molecules are in an ordered, elongated conformation while at higher temperatures gellan gum molecules are in a relatively disordered state. Similar situations were observed by ultrasonic velocity measurement (Tanaka et al., 1993) and circular dichroism (Sakurai et al., 1995). Consequently, the gelation mechanism of gellan gum solutions has been the subject of some controversy, but it is now accepted that gellan gum may undergo a thermally-reversible ordered helix-coil transition, and the junction zones of gellan gum gels are formed by aggregation of double helical gellan molecules, in a way analogous to the gelation of carrageenans. Although gellan gum forms a loose gel by itself (Moorhouse, 1987), the physicochemical properties of gellan gels are influenced strongly by the presence of cations, moreover, they are related to the nature of the cations; divalent cations promote the gelation much more strongly than monovalent cations. Gelation is thus sensitive to cation type, as well as valency (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986 and 1987; Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991; Manning, 1992; Shi, 1990). The temperature of the conformational change of gellan gum determined by circular dichroism or optical rotation is ~30℃. This temperature is controlled by the thermodynamic properties of the systems (temperature, cations, pH) (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986 and 1987; Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991; Manning, 1992; Shi, 1990). Crescenzi et al. (1987) have reported that the conformational transition temperature of gellan gum shifted to higher temperatures with increasing concentration of tetramethylammonium chloride (TMACl). Milas et al.(1990) have suggested from circular dichroism studies, that above the melting temperature (T_m) no ionic selectivity exists for the coil conformation of gellan gum, in contrast, below T_m, a strong aggregation occurs with ionic selectivity yielding an order of effectiveness in promoting aggregation; $K^+ > Na^+ > Li^+ > TMA^+$.

In spite of these extensive studies, the gelation mechanism of gellan gum solutions has not been clarified sufficiently. As is often the case with biopolymers, the results obtained for different samples cannot be compared directly. For example, the molecular weight of gellan gum has been roughly reported to be approximately $1 \sim 2 \times 10^6$ in an earlier study (Glicksman, 1977). However, recent estimations by osmotic pressure and light scattering measurements for soluble tetramethylammonium-type gellan gum have given much smaller molecular weight; $Mn = 5.5 \times 10^4$ (Ogawa, 1993), 2.5×10^5 (Milas et al., 1990), and $Mw = 2.38 \times 10^5$ (Okamoto et al., 1993) 4.33×10^5 (Dentini et al., 1988). Therefore, it is important to compare the experimental results obtained on the same sample especially in the case of biopolymers such as gellan, because even a subtle difference in molecular structure and molecular weight can lead to significant differences in functional properties. It is well known in the history of the development of rheology that the distribution of NBS (National Bureau of Standards, USA) polyisobutyrene has played an important role. Using the same sample, various groups participating in the collaboration could compare their results, and thus made a great contribution to the establishment of a time-temperature superposition principle or a reduced variable method. Based on the same idea, the research group on gellan gum was organised in the Research Group on Polymer Gels affiliated to the Society of Polymer Science, Japan, in order to elucidate the conformation of gellan gum in solution and the gelation mechanism, and develop further industrial applications. Several laboratories have taken part in the collaborative studies on gellan gum using the same sample, and the results were published in a special issue of Food Hydrocolloids. Light scattering (Okamoto et al., 1993), ESR (Tsutsumi et al., 1993), X-ray small angle scattering (Yuguchi, et al., 1993; Yoshida and Takahashi, 1993), osmotic pressure (Ogawa, 1993), ultrasonic velocities (Tanaka et al., 1993) and viscoelastic measurements (Shimazaki and Ogino, 1993; Nakamura et al, 1993; Watase and Nishinari, 1993) have been carried out and much information has been accumulated.

As is well known for polyelectrolytes, the metal content and the type have a strong influence on their solution and gel properties. Unfortunately, the metal contents in the gellan sample used in the above-mentioned collaborative studies were not so low. Therefore, Dr.G.R.Sanderson of Kelco Ltd. was asked to prepare a large quantity of purified sodium type gellan for further collaborative studies. A good sample of sodium form gellan gum with high purity was prepared and distributed to 17 different laboratories.

Finally, an International Workshop on Gellan and Related Polysaccharides (IWGRP) took place in November 1994 in Osaka, Japan, and the aim of this workshop was to facilitate an active communication about the most recent findings on gellan gum or related polysaccharides between scientists, industrialists, food technologists and researchers. E.R.Morris (1994b) discussed the effects of acyl substituents on gellan gum. He reported that the cooling and heating DSC curves for native gellan gum ('high acyl') show endothermic and exothermic peaks at much higher temperatures than for commercial gellan gum ('deacyled') but with no thermal hysteresis between formation and melting. The native gels are weaker than the commercial gellan gum. From this result, Morris suggested that acetyl groups, which are located on the periphery of the helix of gellan gum molecules, prevent aggregation. Rinaudo (1994) has used the purified gellan gum samples, and investigated the molecular weight and conformation of gellan gum using viscosity and light scattering, and determined the persistence length (Lp) based on the Yamakawa model. J.Tang (1994) investigated that rheological properties of gellan gum gels by large deformation tests with tension, compression and torsion modes. Nussinovitch (1994) introduced a new method for food industrial use; he has developed coatings based on gelling agents such as gellan gum, which can provide partial barriers to moisture and gas exchange in fruits and vegetables.

The understanding of the conformation of gellan gum in solution and gelation mechanism has advanced remarkably by using the common gellan gum sample.

We have found that the temperature dependence of the loss shear modulus (G'') for gellan gum solutions shows a step-like change in thermal scanning rheological measurements. We have suggested that this step-like change of G'' can be attributed to coil-helix transition of gellan gum molecules because the molecular ellipticity $[\theta]$ at 204nm (which stays a constant at higher temperatures than 30℃) shows a steep drop at 30℃ observed by circular dichroism (Nakamura et al., 1994). Ohtsuka et al. (1994) have estimated the structural parameters of gellan gum solutions by pulse field gradient stimulated echo NMR methods, and have found that calcium ions are far more effective than potassium ions in changing the structural parameters, which is consistent with our observations by rheology and DSC. Ohtsuka et al. have also found that the diffusional mobility of water in gellan gum gels is much the same as pure water, and this observation is consistent with the conclusion deduced from dielectric measurements by Mashimo et al.. Mashimo et al. (1994) have concluded that most of water in the gellan gum gel is free water, and that free water molecules construct a cluster consisting of 20-30 molecules. Nakajima et al. (1994) have observed gellan gum deposited on highly oriented pyrolytic graphite by scanning tunneling microscopy (STM). They reported that an image of a cross-linking domain, observed in the ambient condition, showed that half a pitch of double helix was ca.2.6nm and that the separation of strands was ca.2.3nm. Since this value of 2.6nm is slightly smaller than that of 2.815nm obtained from X-ray crystallographic study (Chandrasekaran et al., 1988), Nakajima et al suggested that this difference may be caused by the fact that Chandrasekaran's result was obtained in the solid-state whereas Nakajima's was obtained in the gel-state.

In the presence of salts, it has been suggested that the different cations influence the lengths of strands of gellan gum in STM, because the strand length of gellan gum increases in length in the order of no salt$<Na^+<K^+<Ca^{2+}$. This order is in good agreement with that of the effectiveness of the cations in promoting gelation observed in rheological and DSC measurements by us and Izumi et al.. Thus, the differences between the length of strands of gellan gum may influence physicochemical properties of gellan gum.

Ogawa (1994) has found by osmotic pressure measurements that the number average molecular weight M_n obtained at 28℃ was twice as large as that at 40℃, and suggested an association of two molecules at 28℃. From this result, it has been concluded that the disordered structure is a single-coiled chain and the ordered structure is a double-helix. Kubota et al. have discussed the sol-gel transition of gellan gum aqueous solutions in the presence of Ca^{2+} ion based on the percolation model using dynamic and static light scattering, and compared their results with those of Rinaudo et al.. Izumi et al. (1994) have proposed the phase diagrams of gellan gum solutions which are divided into 4 regions using mechanical and DSC methods, and characterized the molecular structure of gellan using an X-ray small angle scattering (SAXS). Kajiwara et al. (1995) have compared potassium type gellan gum with sodium type gellan gum by SAXS, exposing the samples to X-rays as little as possible in order to avoid chain degradation. They found that the crosslinking domains were formed by aggregated double helices, and that the size of domains for potassium type gellan was larger than that for sodium type gellan.

This collaborative work now advances, and there is an urgent need for preparation of more purified samples (sodium type) of gellan with different molecular weights, for distribution to our coworkers with different expertises, in order to achieve a better understanding of the gelation mechanism.

I-2. The Rheological and Thermal Properties of Gellan Gum Aqueous Solutions

MATERIALS AND METHODS

Materials

Gellan samples (in powder form) were kindly given by Kelco Division of Merck & Co. Inc., California, USA in 1993. The contents of the inorganic ions Na^+, K^+, Ca^{2+} and Mg^{2+} were determined by Kelco using an Inductively Coupled Argon Plasma Emission Spectrophotometer (ICP) as Na 3.03%, K 0.19%, Ca 0.11% and Mg 0.02%, respectively.

The powdered sample was dispersed by stirring to swell at 40℃ overnight. The solutions for viscoelastic studies were prepared by stirring at 70℃ for two hours and then heated at 90℃ for one hour to attain a complete transparency. The solutions were then kept at 70℃ to prevent the gelation before each solution was poured onto the plate of the rheological instrument or sealed into the DSC pan.

The concentrations of gellan solutions were changed from 0.2% to 4.2% (w/w).

Rheological measurement

The mechanical spectra measurements were performed with a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd., NJ, USA at various temperatures and frequencies in the linear viscoelastic regime. In this instrument, the complex shear modulus G^* could be detected at the torque measured by the transducer higher than 10^{-3} g·cm. The hot sample solution was poured directly onto the plate of the instrument, using a parallel plate geometry of 50mm diameter, with radial grooves in order to avoid gel slippage. Immediately before each measurement of frequency dependence of the storage shear modulus G' and the loss shear modulus G'', G' and G'' were measured as a function of time at 6.28 rad/s at a constant temperature. The time course of complex shear modulus on gelation induced by quenching to various temperatures from 0℃ to 30℃ was found to attain a plateau value within less than 60min as already shown by Nakamura et al. (1993). Then, frequency sweep experiments (10^{-2} ~10^{1}rad/s) were performed after the accomplishment of the plateau value. Temperature dependence of G' and G'' was observed by cooling the systems from 50℃ to 5℃ and then reheating to 60℃, at the rate of 0.5℃/min, at the constant frequency of 0.1rad/s.

DSC measurement

Differential scanning calorimetry (DSC) measurements were carried out by a Setaram micro DSC-III calorimeter, Caluire, France. Approximately 900mg of the sample solution was sealed into the DSC pan hermetically, and then the pan was accurately weighed. A reference pan was filled with distilled water, to within $\pm 30\mu g$ of the weight of the sample pan. The two pans were then placed inside the calorimeter and heated to 95℃ (or 110℃) and kept for 10min to annihilate the thermal history. Then the temperature was lowered to 5℃ at 0.1~0.5℃/min and raised again at the same rate up to 95℃ (or 110℃). Finally, the temperature was scanned up and down at a fixed constant rate, and the enthalpy profiles were recorded.

RESULTS AND DISCUSSION

Rheological properties

The storage shear modulus G' as a function of time was well approximated by a first-order kinetic equation (data not shown) as has been shown for gellan (Nakamura et al., 1993), for soybean protein (Nishinari et al., 1991) and for casein (Tokita, 1989). After the accomplishment of plateau values (ca. 60min), the frequency dependence of storage modulus G' and loss modulus G" was observed.

Figure I-2-1 (a)~(c) shows the frequency dependence of G' and G" for 1.0%, 2.0% and 3.0% gellan gum solutions at various temperatures. It is clear that, at any temperature from 30℃ to 0℃, the viscoelastic behaviour of a 1% gellan gum solution [Figure I-2-1(a)] is typical of a dilute polymer solution with G'<G" throughout the accessible frequency range, and both G' and G" are strongly frequency dependent. For a 2% gellan gum solution [Figure I-2-1(b)], the behaviour at 30℃ is typical of a dilute solution, however, the behaviour at lower temperatures (25℃, 15℃) is typical of a concentrated polymer solution. In concentrated polymer solutions, the response is predominantly liquid-like, i.e., G" is larger than G' and both moduli increase with increasing frequency at low frequencies, whilst the behaviour approaches that of a solid, i.e., G' is larger than G" and both moduli become less frequency dependent at higher frequencies. The molecular chains can disentangle and rearrange during the long period of oscillation, then G"

predominates G' at lower frequencies in concentrated solutions, whilst at higher frequencies disentanglement cannot occur during the short period of oscillation, resulting G'>G" because the entanglement plays a role of temporary cross-linking junction zones. The frequency dependence at 0℃ was different; G' was larger than G" with little frequency-dependence throughout the accessible frequency range. This behaviour may be classified rheologically as that of 'weak gel' as described in section 2 of Introduction.

For a 3% gellan gum solutions [[Figure I-2-1(c)], the behaviour at 30℃ is typical of a concentrated polymer solution, however, the behaviour is different at lower temperatures than 25℃, where G' is larger than G" throughout the experimentally-accessible frequency range, and both moduli are little dependent on frequency, as would be expected for a weak gel.

Figure I-2-2(a)~(c) shows the temperature dependence of G' and G" during cooling or heating process for 1%, 2% and 3% gellan gum solutions without salt at 0.1 rad/s at a cooling or heating rate of 0.5℃/min. For a 1.0% solution of gellan gum [Figure I-2-1(a)], G' was too small to be detected in both cooling and heating process, however, G" could be detected at temperatures lower than 50℃ in both cooling and heating processes and showed a step-like change at 30℃. It was found that the temperature dependence of G" for gellan gum solutions of a concentration lower than 2.0% showed one step-like change at a certain temperature (not all the data are shown). It seems that the temperature at which G" drastically changes should be a kind of transition temperature. As illustrated in the frequency dependence of both moduli for a 1% gellan gum solution [Figure I-2-1(a)], at any temperature from 30℃ to 0℃, the viscoelastic behaviour was typical of a dilute polymer solution. As concluded from the frequency dependence of both moduli described above, at any temperature from 30℃ to 0℃, the viscoelastic behaviours of gellan gum solutions of a concentration lower than 2.0% are characteristic to a dilute or a concentrated polymer solution. Therefore, the transition observed in the temperature dependence of G" for gellan gum solution of a concentration lower than 2.0% should not be attributed to the sol-gel transition, but may be induced by coil-helix transition, and the temperature will be written as Tch hereafter. At lower concentrations of gellan gum molecules, the helix formation and its partial aggregation may form a certain ordered structure, but does not lead to the gel formation because the number of helical aggregates is not sufficient to prevail the whole space. Therefore, the apparent plateau at lower temperatures should not be identified as the rubber-like plateau but it is similar to the second plateau for suspensions discussed by Onogi and Matsumoto (1970). The difference between G" at lower and higher temperature sides at the midpoint temperature of transition measured by the ordinate unit in the thermal scanning rheological measurements was called the relaxational strength ΔG in this study [Figure I-2-2(a)]. The relaxational strength ΔG is usually defined for storage modulus, or generally speaking, the relaxational strength is defined as the difference or the gap at the transition of the real part of a complex response function as a function of frequency or as a function of temperature, between the values of both sides at the transition (Wada, 1971). However, G" could be detected at a wider temperature range than G' in the present study, and the gap of G" at the transition is defined as the relaxational strength. ΔG at T_{hc} increased with increasing concentration of gellan gum in the present study. However, the thermal behaviour of gellan gum solutions of a concentration higher than 2.0% showed two step-like changes in the present study. For a 2.0% gellan gum solution [Figure I-2-2(b)], G' could be detected at 34℃ on cooling and then gradually increased, and moreover it also increased rapidly around 10℃ and G' became significantly larger than G" at temperatures lower than the cross-over temperature. G" for a 2.0% gellan gum solution showed two step-like changes at both 35℃ and 10℃. For a 3.0% gellan gum solution [Figure I-2-2(c)], the cross-over of G' and G" was observed at 28℃ and this temperature was significantly higher than that for a 2.0% gellan gum solution [Figure I-2-2(b)]. For a 3.0% gellan gum solution [Figure I-2-2(c)], the temperature at which G" showed the first step increase in the cooling process was also slightly higher than that for a 2.0% gellan gum solution. Such a behaviour as showing two step-like changes of G" was observed for gellan gum solutions of a concentration higher than 2.0% in the present work.

The elastic modulus of gellan gels will increase with increasing number of elastically active network chains or with increasing number of junction zones. The two step increase in elastic modulus should then be attributed to the two step increase in the number of junction zones. The higher temperature process may be induced by the helix-coil transition at T_{hc}, which corresponds to the temperature at which G'' for gellan gum solutions of low concentrations showed one step-like change, but the subsequent lower temperature process at which G' and G'' showed a cross-over may be attributed to the sol-gel transition. Since the gellan gum solutions whose concentrations are lower than 2.0% showed a typical mechanical spectrum for concentrated polymer solutions at any temperature from 30℃ to 0℃, the transition observed for these solutions cannot be attributed to a gel-sol transition but to a helix-coil transition, as mentioned above. However, as shown in the frequency dependence of both moduli [Figure I-2-1(b)], the viscoelastic behaviour for a 2.0% gellan gum solution is typical of a dilute solution at 30℃ and is typical of a concentrated polymer solution at 25℃ or 15℃, however the behaviour changed to that of a weak gel at 0℃. For a 3.0% gellan gum solution [Figure I-2-1(c)], although the viscoelastic behaviour remained typical of a concentrated solution at 30℃, the behaviour tended towards that of a weak gel at temperatures lower than the cross-over temperature of both moduli (27℃). Therefore, in concentrated solutions (> 2.0%), where the number of aggregates of helices exceeds a critical value on cooling, the rheological behaviour changes from sol to gel, and this appears as the second step increase of G'' shown in this figure. However, it is worthy of note that the temperature at which G' becomes larger than G'' was slightly lower than the temperature at which G'' shows the second step increase. Hence, G'' for a 1.9% gellan gum solution (Figure I-2-3) showed the second step increase around 8℃ in the cooling process, however G'' remained slightly larger than G' down to 5℃. In the case of the frequency dependence of both moduli (data not shown), the viscoelastic behaviour for a 1.9% gellan gum solution was typical of a dilute solution at 10℃, and was typical of a concentrated polymer solution at 5℃, however, the behaviour changed to that of a weak gel at 0℃. Therefore, the cross-over temperature of G' and G'' at 0℃ (Figure I-2-3) may be assigned as a sol-gel transition temperature, and it will be written as T_{sg} hereafter.

Figure I-2-4(a)~(c) shows the temperature dependence of G' and G'' during cooling or heating process for 3.2%, 3.3% and 3.5% gellan gum solutions without salt at 0.1 rad/sec at a cooling or heating rate of 0.5℃/min. The thermal behaviour for a 3.2% gellan gum solution [Figure I-2-4(a)] was evidently distinguished from that for gellan gum solutions of a concentration lower than 3.2% (Figure I-2-2 and I-2-3); G'' showed two step increase at both 40℃ and 33.1℃ in the cooling process, however it showed two step decrease at 39.1℃ and 48.0℃ in the heating process, which indicated that the thermal hysteresis was greatly modified compared to that for gellan gum solutions of a concentration lower than 3.2%. Moreover, the temperature at which G'' showed the first step decrease in the heating process almost coincided with the lower endothermic peak temperature in the heating DSC curve (Figure I-2-6), and the temperature at which G'' showed the second step decrease almost coincided with the higher temperature endothermic peak, as will be discussed later. For a 3.5% gellan gum solution [Figure I-2-4(c)], G'' showed one step increase at 40℃ in the cooling process, in other words, T_{hc} corresponding to the helix-coil transition and T_{sg} corresponding to the sol-gel transition were observed at the same temperature. Therefore, this suggested that the coil-helix transition occurs concurrently with the sol-gel transition since the number of helices formed on cooling is enough to prevail the whole space and to form a three dimensional network. Although G'' for a 3.5% gellan gum solution showed two step decrease in the heating process, the temperature where it showed the first step decrease was almost similar to that for a 3.2% or 3.3% gellan gum solution [Figure I-2-4(a) or (b)], but the temperature at which it showed the second step decrease significantly shifted to higher temperatures with increasing concentration of gellan gum. Moreover, the relaxational strength at the temperature of first step decrease in the heating decreased but that at the temperature of the second step decrease increased with increasing concentration of gellan gum.

As found in the frequency dependence of both moduli, the viscoelastic behaviour for a 3.2, 3.3 or 3.5% gellan gum solution tended towards that of a weak gel at any temperature from 30 to 0℃ (data not shown).

Figure I-2-5 shows the temperature of the first step increase of G'' in the cooling process (T_{hc}) and the temperature of the second step increase of G'' (T_{sg}) for gellan gum solutions as a function of concentration of gellan gum from 0 to 3.5%. As shown in this figure, both T_{hc} corresponding to the helix-coil transition and T_{sg} corresponding to the sol-gel transition significantly shifted to higher temperatures with increasing concentration of gellan gum. Moreover, the difference between T_{hc} and T_{sg} gradually decreased with increasing concentration of gellan gum, and eventually, for a 3.5% gellan gum solution, the helix-coil transition and the sol-gel transition occurred almost concurrently.

DSC

Figure I-2-6 shows cooling and heating DSC curves of gellan gum solutions of various concentrations without salt at cooling or heating rate of 0.5℃/min. The cooling curve for a 1% gellan gum solution without salt showed a single exothermic peak at 29.0℃, and the heating curve showed a single endothermic peak at 30.5℃. The endothermic peak temperature in heating DSC curves and the exothermic peak temperature in cooling DSC curves will be called melting temperature T_m and setting temperature T_s hereafter. Thus, T_m was slightly higher than T_s for 1% gellan gum solutions, as has been observed for many thermoreversible gels (Guenet, 1992).

Figure I-2-7 shows the scan rate dependence of T_s or T_m for 1% gellan gum solutions. T_s or T_m varied linearly with scan rate, and could therefore be extrapolated to zero scan rate. It has been reported that the extrapolated value of T_s at zero scan rate from cooling curves was in good agreement with the corresponding value of the conformational transition temperature determined from circular dichroism or optical rotation (Manning, 1992). In the present work, the values of individual T_s and T_m for 1% gellan gum solutions extrapolated to essentially the same value 28.6℃ at zero scan-rate (Figure I-2-7), which agreed fairly well with a result reported by Manning (1992). This indicates that there is no detectable thermal hysteresis between formation and melting of the ordered structure (Manning, 1992). The cooling curve for a 2.0% gellan gum solution showed a main exothermic peak at 33.9℃ and another small peak at 95℃ while the heating curve showed only a main endothermic peak at 35.4℃. As shown in the rheological measurement, G'' for a 2.0% gellan gum solution showed two step-like changes at both 35℃ and 10℃. Although the subsequent lower temperature process at which G' and G'' showed a cross-over may be attributed to the sol-gel transition, this transition around 10℃ was not observed in the DSC measurement. Therefore, at low concentration of gellan gum, these exothermic and endothermic peaks seem to be attributed to the helix-coil transition of gellan gum molecules and the subsequent aggregation of these helices. Both exothermic peak temperature T_s and endothermic peak temperature T_m shifted to higher temperatures and both exothermic and endothermic enthalpies increased with increasing concentration of gellan gum, which corresponds well with rheological results. Moreover, the temperature T_{hc} corresponding to the helix-coil transition in rheological measurement was found to be inbetween the exothermic and endothermic peak temperatures T_s and T_m observed in cooling and heating DSC curves (Figures I-2-2, I-2-4 and I-2- 6). However, for a gellan gum solution of a concentration higher than 3.2%, the endothermic peak in the heating curve splitted into two peaks, while the cooling curve showed a main exothermic peak and other small exothermic peaks at higher temperatures. As mentioned above, the detectable splitting in heating DSC curves and the large hysteresis in elastic moduli inbetween heating and cooling process were observed for gellan gum solutions >3.2%. It is clear that the lower temperature endothermic peak corresponds with the first step decrease of G" in heating process of thermal scanning rheological measurement, and the higher temperature endothermic peak corresponds with the second step decrease of G". Moreover, as illustrated in this figure, the enthalpy determined from the lower temperature endothermic peak decreased with increasing concentration of gellan gum, and in contrast, the enthalpy determined from the higher temperature endothermic peak increased with increasing concentration of gellan gum, which was in good agreement with that in the temperature dependence of elastic moduli shown above [Figure I-2-4(a)~(c)]. It was thus considered that gellan gum solutions whose concentration was higher than 3.2% formed the gel network containing thermally-stable junction zones. Manning (1992) suggested that the lower temperature peak may be caused by the melting of unaggregated helices while the higher temperature peak may be caused by the melting of aggregated helices. However, our interpretation is different from theirs.

We propose the following interpretation of rheological and thermal results for gellan gum solutions without salts, as outlined schematically in Figure I-2-8. In the cooling process, although the individual helices of gellan gum molecules are formed at a certain temperature and then these helices gradually aggregated with decreasing temperature, at lower concentration of gellan gum, the helix formation and its partial aggregation may form a certain ordered structure, but does not lead to the gel formation, so that the temperature dependence of loss modulus for these solutions showed one step-like change at the coil-helix transition temperature T_{ch}. However, for concentrated gellan gum solutions (>2.0%), where the number of aggregates of helices exceeds a critical value on cooling, the rheological behaviour changes from sol to gel, and this appears as the second step increase of G" at the sol-gel transition temperature T_{sg}. In the case of concentrated gellan gum solutions (>3.2%), the first step increase of G" in the cooling process (a) almost coincided with the second step decrease of G" in the heating process (d), and the second step increase of G" in the cooling process (b) almost coincided with the first step decrease of G" in the heating process (c). With more progressive increase of concentrations of gellan gum, the individual helices were formed and immediately the aggregation of helices occurred in the cooling process, so that for 3.5% gellan gum solution, G" showed one step increase in the cooling process, as shown in rheological measurement [Figure I-2-4(c)]. However, in the heating process, the temperature dependence of G" for concentrated gellan gum solutions whose concentration was higher than 3.2% showed a larger hysteresis, and these solutions showed two endothermic peaks in the heating DSC curve. Therefore, in the heating process, the lower temperature process may be caused by the separation of the aggregated helices into single helices, while the higher temperature process may be induced by the helix-coil transition. It was concluded from both rheological and thermal results that for concentrated gellan gum solutions(>3.2%), the conformational transition temperatures in the heating process did not coincide with those in the cooling process.

Figure I-2-9 shows the exothermic peak temperature T_s and endothermic peak temperature T_m for gellan gum solutions as a function of concentration of gellan gum from 0 to 4.2%. The experimental result that the endothermic peak temperature T_m in heating DSC curves is higher than the exothermic peak temperature T_s in cooling DSC curves is commonly observed for many thermoreversible systems, as mentioned above. As shown in Figure I-2-7, however, the extrapolation of scan rate to zero will make this difference between T_m and T_s negligible for gellan gum solutions of lower concentrations. It is clearly demonstrated that both T_s and T_m shifted to higher temperatures with increasing concentration of gellan gum, which corresponds well with rheological results. Moreover, the temperature T_{hc} corresponding to the helix-coil transition in rheological measurement was found to be inbetween the exothermic and endothermic peak temperatures T_s and T_m observed in cooling and heating DSC curves (Figure I-2-5 and Figure I-2-9). However, the lower temperature endothermic peak for concentrated gellan gum solutions (>3.2%) maintained plateau, and as a result, for concentrated gellan gum solutions (>3.8%), the exothermic peak temperature became slightly higher than the lower endothermic peak temperature. It, thus, seems that the gelation mechanism for gellan gum solutions of higher concentrations becomes more complicated.

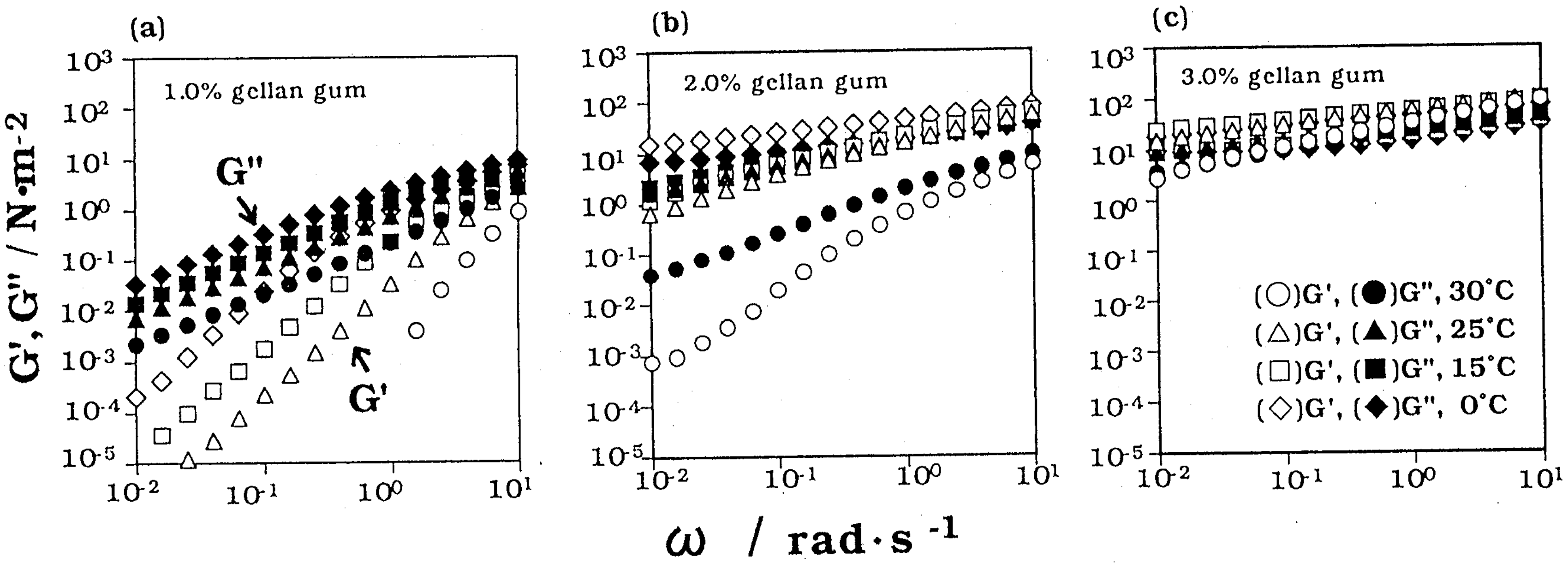

Fig. I-2-1 Frequency dependence of storage modulus G' and loss modulus G" for 1%, 2% and 3% gellan gum solutions at various temperatures. (○)G', (●)G", 30℃; (△)G', (▲)G", 25℃; (□)G', (■)G", 15℃; (◇)G', (◆)G", 0℃.

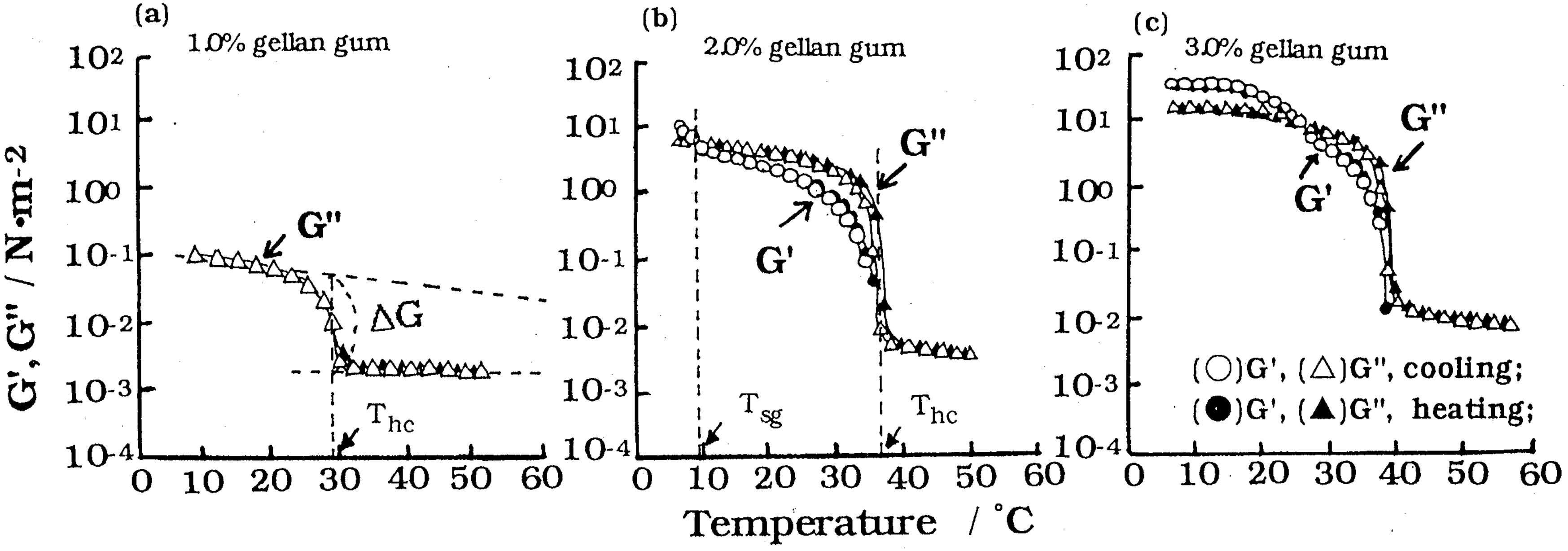

Fig. I-2-2 Temperature dependence of storage modulus G' and loss modulus G' during cooling or heating process for 1%, 2% and 3% gellan gum solutions. (○)G',(△)G",cooling; (●)G',(▲)G",heating; cooling and heating rate, 0.5℃/min; freq.ω=0.1rad/sec.

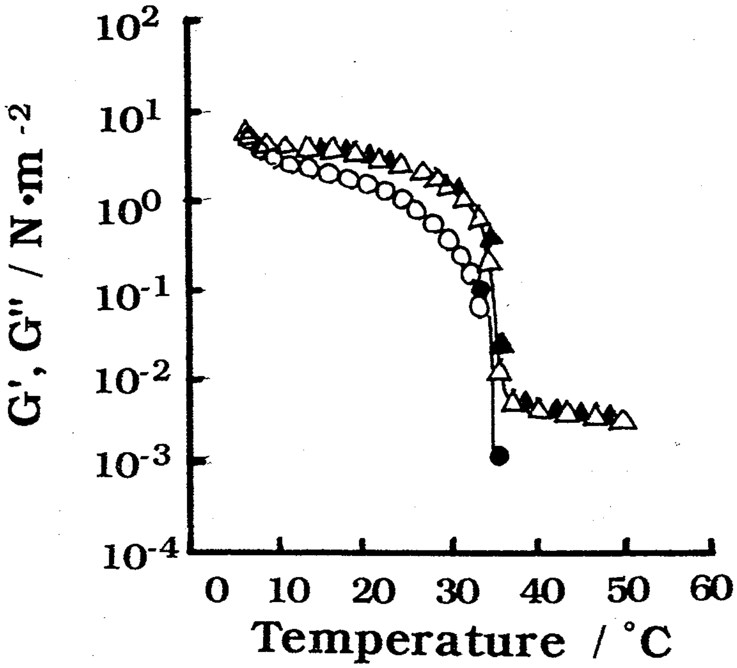

Fig. I-2-3 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 1.9% gellan gum solution. (○)G',(△)G",cooling; (●)G',(▲)G",heating; cooling and heating rate, 0.5℃/min; freq.ω=0.1rad/sec.

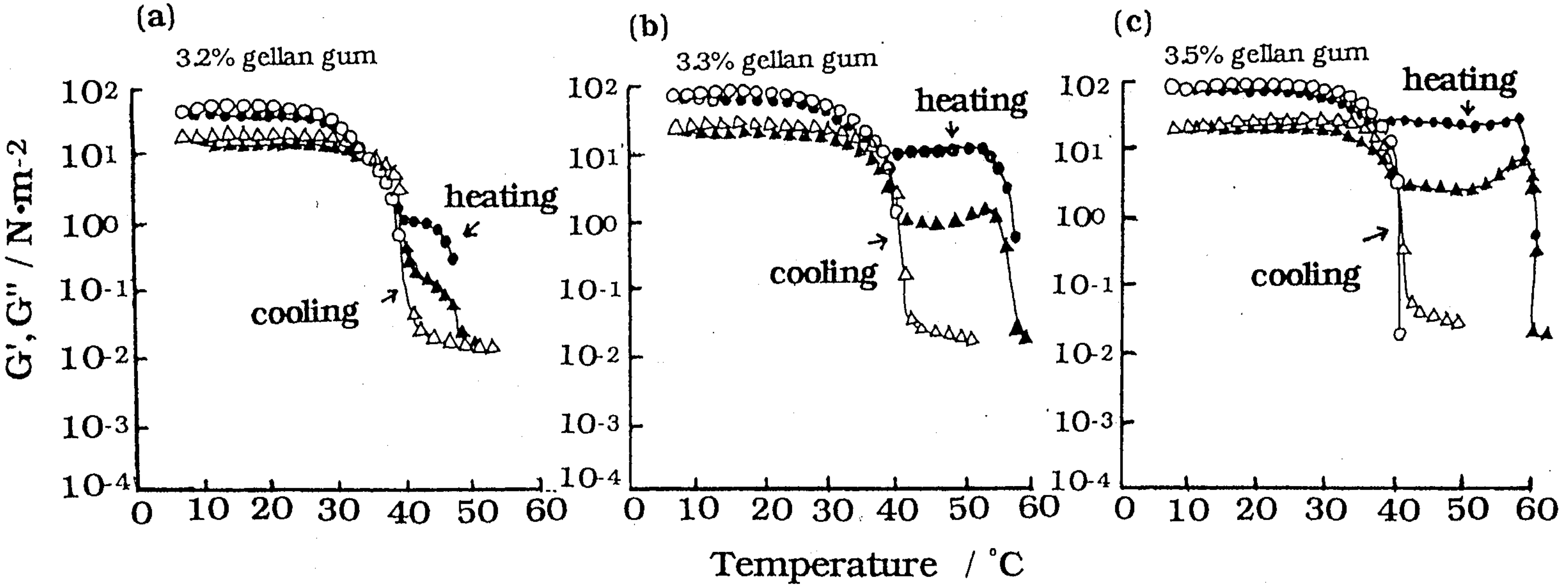

Fig. I-2-4 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for 3.2%, 3.3% and 3.5% gellan gum solutions. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s;

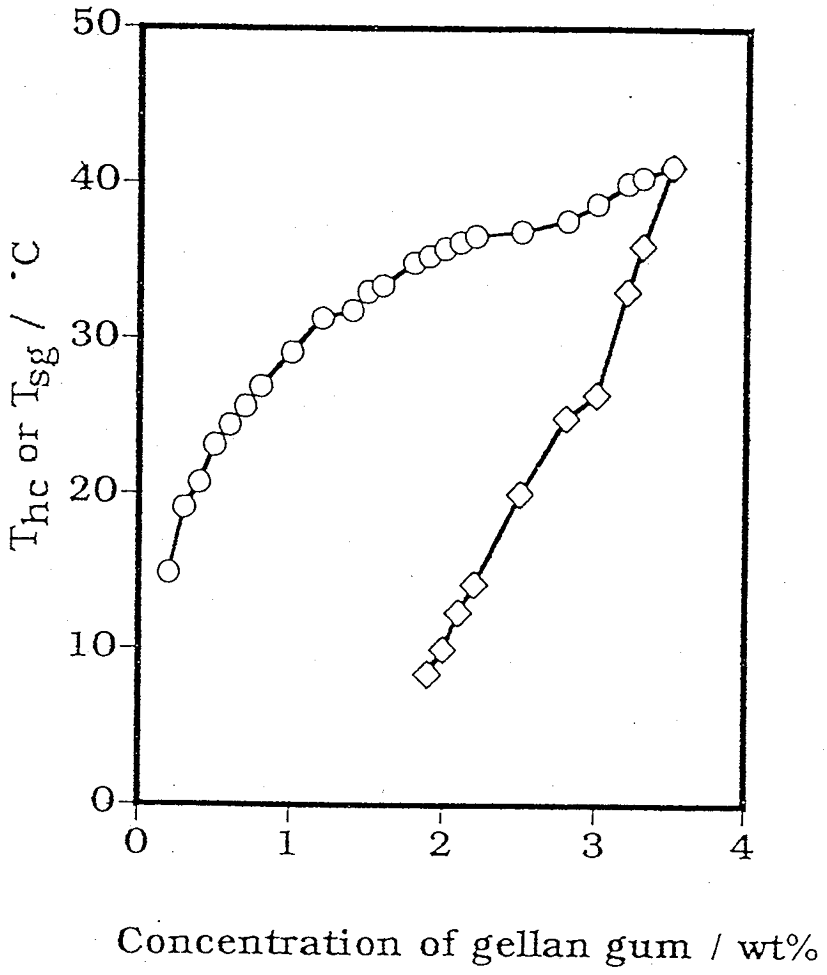

Fig. I-2-5 First step increase temperature of G", T_{hc} (○) and the second step increase temperature of G", T_{sg} (◇) for gellan gum solutions in the cooling process as a function of concentration of gellan gum from 0 to 3.5%.

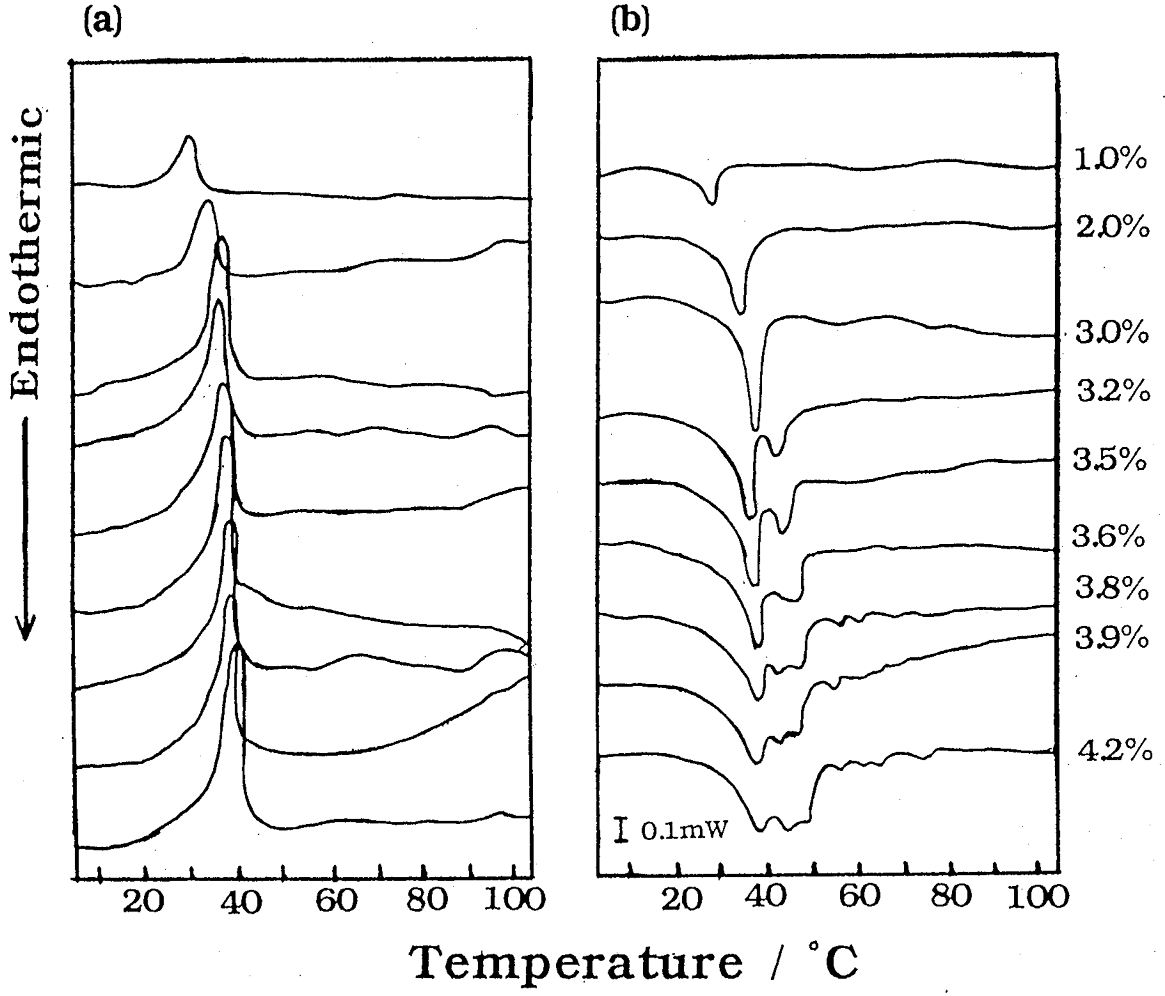

Fig. I-2-6 Cooling (a) and heating (b) DSC curves for gellan gum solutions of various concentrations. Figures beside each curve represent the concentration of gellan gum. Cooling and heating rate:0.5℃/min.

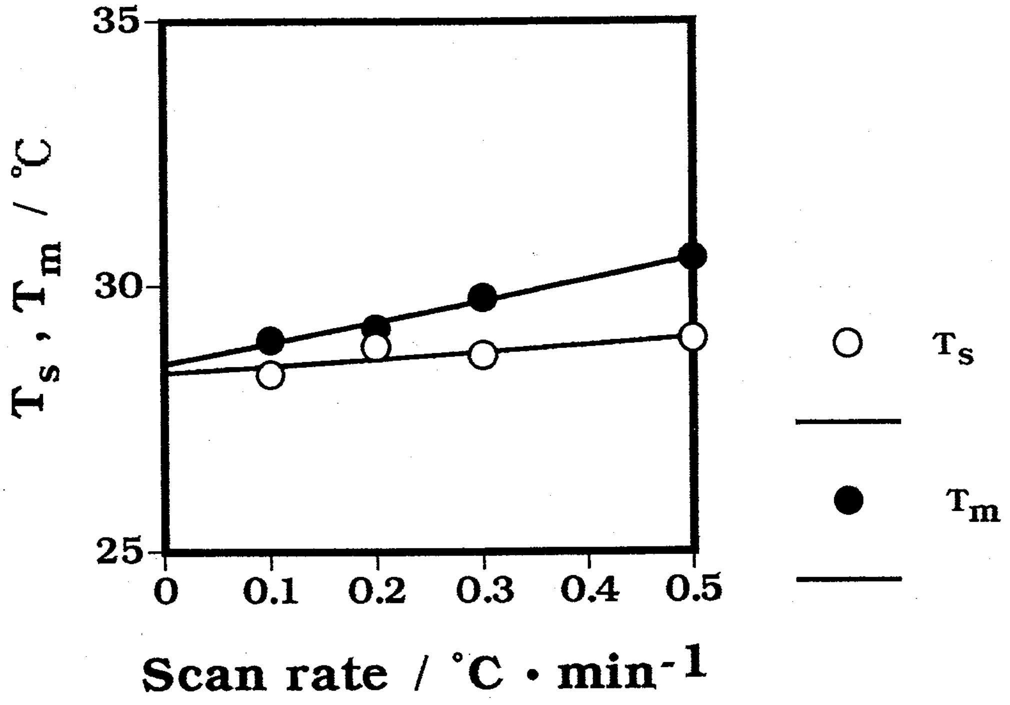

Fig. I-2-7 Scan rate dependence of the setting temperature T_s or the melting temperature T_m for 1% gellan gum solutions.

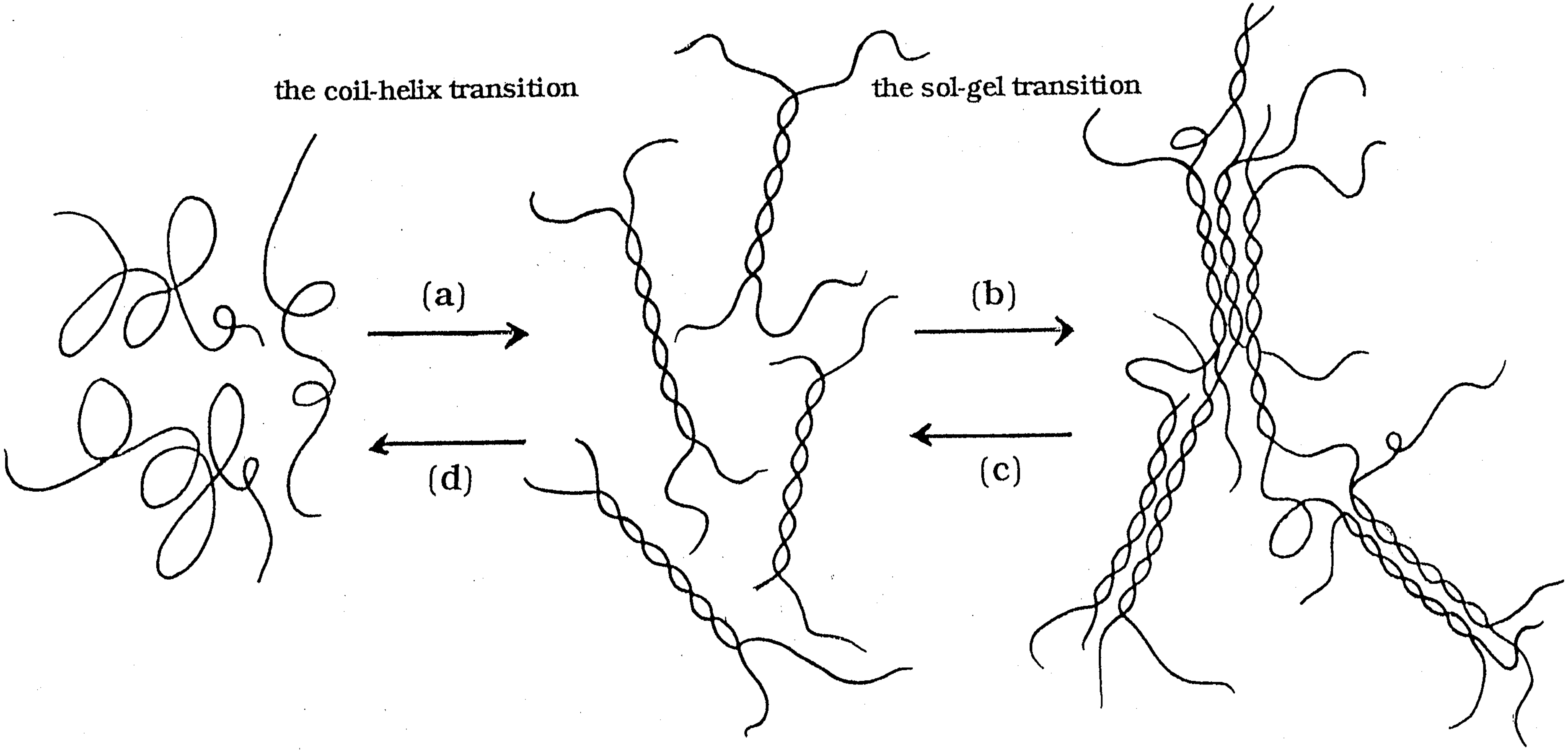

Fig. I-2-8 Schematic representation of the structure of gellan gum, showing various processes during cooling or heating process.
(a) the first step increase of G" in the cooling process
(b) the second step increase of G" in the cooling process
(c) the first step decrease of G" in the heating process
= the lower temperature process in the heating DSC curve
(d) the second step increase of G" in the heating process
= the higher temperature process in the heating DSC curve

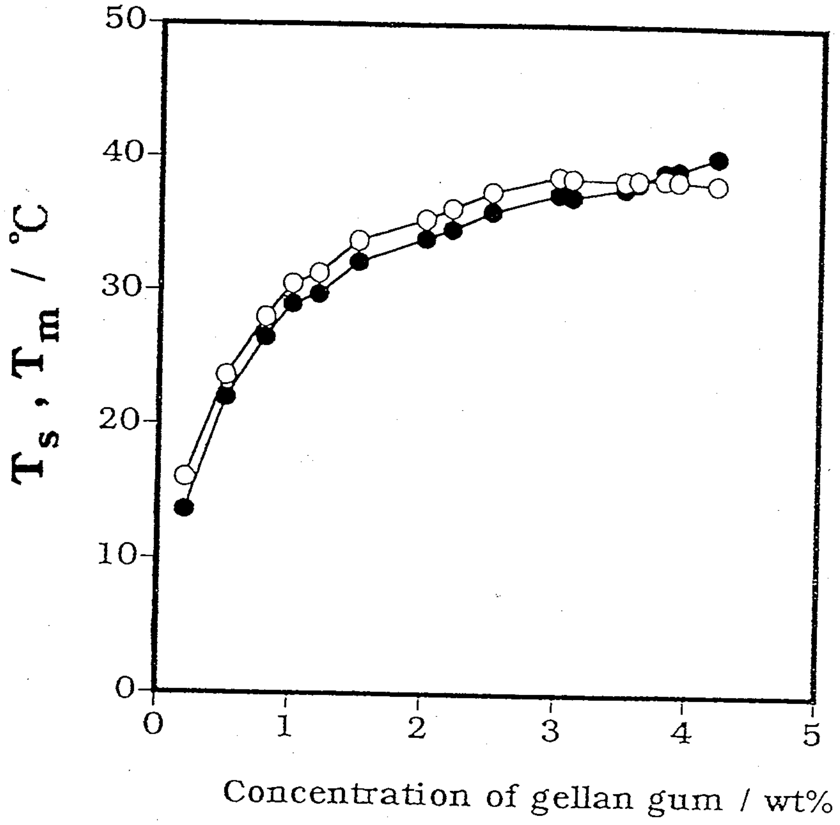

Fig. I-2-9 Exothermic peak temperature T_s(●) and endothermic peak temperature T_m(○)for gellan gum solutions as a function of gellan gum concentration.

II. Effects of Salts on Sol-Gel Transition in Gellan Gum Aqueous Solutions

MATERIALS AND METHODS

Gellan gum used in this chapter was the same as described in Chapter I. Salts, NaCl, KCl, $CaCl_2$ and $MgCl_2$ used in this study were of the extra fine grade reagents (Wako Pure Chemical Industries Ltd., Osaka, Japan), and were used without further purification.

The gellan gum solutions were prepared in the same way as described in section 2 of Chapter I. The concentration of gellan solutions were fixed as 1% or 2% (w/w). For samples containing salts, the concentration of NaCl or KCl in solutions varied from 5 to 100mM and that of $CaCl_2$ or $MgCl_2$ ranged from 0.43 to 6.8mM.

Methods of rheological and DSC measurements were described in section 2 of Chapter I.

RESULTS AND DISCUSSION

Figure II-1 shows the frequency dependence of G' and G'' for 1% gellan gum solutions containing NaCl of various concentrations at various temperatures. Upon addition of 5mM NaCl [Figure II-1(a)], the viscoelastic behaviour remained typical of a dilute solution at any temperature from 0℃ to 30℃, and moreover, G' was slightly smaller than that of 1% gellan solution without salt. Upon addition of 10mM or 20mM NaCl at any temperature [Figure II-1(b),(c)], G' became significantly larger than those without salt. The spectrum in the presence of 20mM NaCl at 0℃ resembled that of a concentrated solution or an entanglement system because G' approached G'' at lower frequencies and in fact, G' and G'' actually showed a 'cross-over' at a lower frequency 6.03×10^{-3} rad/s (data not shown). Upon addition of 25mM NaCl (Figure II-1(d)), G' was larger than those without salt, however, at 15℃, the effects of NaCl varied depending on frequency; at lower frequencies than 10^{-1} rad/s, G′ was too small to be detected although G' of 1% solution without salt was detectable [Figure I-2-1(a)]; in contrast, at higher frequencies, G' was larger than those without salt. Moreover, the behaviour in the presence of 25mM at 0℃ tended towards that of a concentrated solution, and G' and G'' showed a 'cross-over' at a higher frequency ($\omega=$

5.70×10^{-2} rad/s) than $\omega = 6.03 \times 10^{-3}$ rad/s at which the solution in the presence of 20mM NaCl showed a cross-over of G' and G". Upon addition of 50mM NaCl [Figure II-1(e)], G' at 25℃ was significantly smaller than those without salt throughout the accessible-frequency range, and at 15℃, G' was too small to be detected at low frequencies. However, at 0℃, the behaviour in the presence of 50mM tended towards that of a weak gel. Upon addition of 75mM NaCl [[Figure II-1(f)], the behaviour became strikingly different; G' was much larger than G" throughout the experimentally-accessible frequency range and both moduli were essentially independent of frequency, as would be expected for an elastic gel. The values of G' and G" for 1% gellan gum solution with 75mM NaCl were significantly larger than that of 2% or 3% gellan gum solution without salt [Figure II-1(b) and(c) in Chapter I]. This confirmed that the rheological properties of gellan gum solutions were influenced strongly by the presence of salt (Tako et al., 1989; Moritaka et al., 1991; Watase and Nishinari, 1993; Manning, 1992; Chapman et al., 1900; Robinson, 1991). Moreover, at any temperature from 30℃ to 0℃, particularly even at a higher temperature such as 30℃, the behaviour tended towards that of an elastic gel. Thus, it seems that an elastic gel network is formed even at a higher temperature as 30℃ in the presence of sufficient NaCl. It was shown that the conformational transition temperature shifted to higher temperatures with increasing concentration of tetramethylammonium chloride (TMACl) (Crescenzi, 1988). It is, therefore, suggested that the conformational transition temperature shifted to a temperature higher than 30℃ on addition of 75mM NaCl from the results shown in Figure II-1(f).

Values of G' for 1% gellan gum solutions at a constant temperature and at a constant frequency as a function of NaCl concentration showed a complicated behaviour. G' at 0℃ decreased at all the frequencies examined on addition of 5mM NaCl [Figure II-1(a)] as mentioned above, and it increased with increasing concentration of NaCl up to 20mM [Figure II-1(b) and (c)], but it decreased on addition of 25mM NaCl [Figure II-1(d)], and then it increased with increasing concentration of NaCl [Figure II-1(e) and (f)]. The more detailed examination of the dependence of G' for 1% gellan gum solutions at 30℃ and 0℃ (at 0.1rad/s) on the concentration of NaCl is shown in Figure II-2. G' as a function of NaCl concentration showed a complicated curve : it resembles the sinusoidal curve superposed to a straight line with a positive slope. The increase in G' with increasing sodium ion concentration may be understood by the electrostatic shield of carboxylic ions in gellan molecules, however, the subsequent decrease in G' with increasing sodium ion concentration is not understood by such a simple mechanism. The storage Young's modulus E' of κ-carrageenan gels was shown to increase by the addition of alkali metal or alkali earth metal salts but the excessive addition of these salts decreased E' (Watase and Nishinari, 1982, 1986 and 1988, Watase et al., 1990ab). In previous studies, not only the maximum of the elastic modulus of κ-carrageenan gels as a function of the concentration of the added salt but also the multiple maxima were found with alkali metal salts (Watase and Nishinari, 1982) or with tetra-alkyl ammonium bromide (Watase et al., 1990a) or with ammonium salts (Watase et al., 1990b). However, the mechanism of this phenomenon has not been understood. The increase in E' was attributed to the shield of electrostatic repulsion of sulfate groups in κ-carrageenan molecules, however, the reason why E' decreased by the excessive addition of the salts remained unclarified. The increase in G' of gellan solutions by the addition of more than 10mM NaCl may be attributed to the shield of electrostatic repulsion between carboxyl groups in gellan molecules, but at the same time NaCl also changes the structure of water as a solvent. Therefore, the occurrence of increase and decrease with increasing concentration of salt may be governed by these multiple factors, and cannot be explained by a single factor. This should be explored more quantitatively in the near future.

Figure II-3 shows the frequency dependence of G' and G'' for 1% gellan gum solutions in the presence of 50mM NaCl at 25℃ [Figure II-3(a)] or 0℃ [Figure II-3(b)] compared to those without salt. As is clearly seen in these figures, 50mM NaCl decreased both moduli at 25℃ whilst the same amount of NaCl increased both moduli at 0℃. It is thus likely that even if the same concentration of NaCl was added to gellan gum solutions, NaCl plays conflicting roles to rheological properties of gellan gums, depending on temperature. This reason would be explained as follows. The electrostatic repulsion between carboxyl groups in gellan molecules is shielded by increasing the concentration of cations, and as a result, the helix formation and aggregation of polymers are promoted (Manning, 1992; Shi, 1990;

Crescenzi and Smidsrod, 1987; Crescenzi et al., 1986, 1988, Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991). However, in the case of the coil conformation for polymers, the electrostatic shield leads to the reduction of coil dimensions (Robinson et al., 1991) as in the case of hyaluronic acid solutions (E.R.Morris et al., 1980; Kobayashi et al., 1994). The conformational transition of the thermoreversible gels is controlled by temperature (Crescenzi et al., 1988), and gellan gum also shows the change from coil to helical conformation with temperature change (Manning, 1992; Shi, 1990; Crescenzi and Smidsrod, 1987; Crescenzi et al., 1986, 1988, Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991). It was shown that tetramethylammonium chloride increased the intrinsic viscosity [η] of gellan gum solution at 25℃ whilst the same salt decreased [η] at 40℃, and it was suggested that at lower temperatures gellan molecules are in an ordered, elongated conformation whilst at higher temperatures gellan molecules are in a relatively disordered state (Crescenzi et al., 1988). Similar situations were observed by ultrasonic velocity measurements (Tanaka et al., 1993). Therefore, we believe that even if the same amount of cations was added to a gellan gum solution, both G' and G'' increased at a lower temperature where gellan molecules take helical conformations whilst both moduli decreased at a relatively higher temperature where gellan molecules take coil conformations.

Figure II-4 shows the frequency dependence of G' and G'' for 1% gellan gum solutions containing KCl of various concentrations at various temperatures. Upon addition of 5mM KCl [Figure II-4(a)], G' and G'' showed a similar behaviour to that of a 1% solution without salt, although G' at 25℃ was slightly smaller and G' at 0℃ was slightly larger. Upon addition of 10mM KCl [Figure II-4(b)], G' was significantly larger than those without salt, especially, at 0℃ G' and G'' showed markedly less frequency dependence without cross-over at lower frequencies, (~10^{-3} rad/s), which may be an indication of some weak intermolecular association within the system. Upon addition of 20mM KCl [Figure II-4(c)], G' was larger than those without salt, however, at 25℃, G' was too small to be detected at low frequencies. Upon addition of 25mM [Figure II-4(d)], at 30, 25 and 15℃, G' was significantly smaller than those without salt. However, G' at 0℃ was significantly larger than those without salt, and the behaviour tended towards that of a weak gel. Therefore, effects of KCl were different at lower temperatures and at higher temperatures. This result showed a similar tendency to that of 1% solutions with 50mM NaCl [Figure II-1(e)], although the concentration of added KCl was lower than that of NaCl. Both G' and G'' became too small to be detected above 30℃ in the presence of KCl less than 25mM KCl. However, upon addition of 30mM KCl [Figure II-4(e)], at any temperature from 30℃ to 0℃, particularly at higher temperature, larger values of G' and G'' were observed and those curves were little dependent on frequency, indicating that a gel network was formed even at a higher temperature such as 30℃. This is a similar tendency to that of a 1% gellan gum solution with 75mM NaCl [Figure II-1(f)], although G' and G'' of a 2% gellan gum solution with 30mM KCl were smaller than those of a 1% gellan gum solution with 75mM NaCl. Upon addition of 50mM KCl [Figure II-4(f)], the viscoelastic behaviour tended towards that of an elastic gel at any temperature from 30℃ to 0℃. On the other hand, it is noticeable that the minimum salt concentration necessary to make an elastic gel is lower for KCl (50mM) than for NaCl (75mM). Here, G' was greater than G'' throughout the accessible frequency range and both moduli were essentially independent of frequency. However, measured values of G'' showed a shallow minimum centered at 10^{-1}~$1.0\mathrm{rad\cdot s^{-1}}$ as described in Section 2 of Introduction.

As was found in the gellan gum solutions containing NaCl (Figure II-1), values of G' for 1% gellan gum solutions as a function of KCl concentration also showed a complicated behaviour. G' at 0℃ increased at all the frequencies by the addition of 5mM KCl (Figure II-4(a)), and 10mM KCl (Figure II-4(b)) as mentioned above. However, on addition of 20mM KCl (Figure II-4(c)) G' decreased, and on addition of 30mM KCl (Figure II-4(e)) it decreased, and on addition of 50mM KCl (Figure II-4(f)) it increased again. This complicated behaviour cannot be explained at present, and should be explored in the future.

The comparison of Figure II-1 with Figure II-4 shows that the viscoelastic behaviour of 1% gellan gum solutions was more influenced by KCl than by NaCl. The reason why KCl can influence the viscoelastic behaviour of gellan gum solutions more effectively than NaCl is interpreted on the basis that the cations such as Na^+ are the structure ordering ions for water, and the cations such as K^+ belong to the structure disordering ions (Uedaira and Ohsaka, 1990). Therefore, the latter group can shield the electrostatic repulsion of carboxyl side groups in the gellan gum molecules more directly than the former group, just as in the case of the addition of salts to κ-carrageenan; the structure disordering ions increased E' much more than the structure ordering ions by shielding the electrostatic repulsion of sulfate groups (Watase and Nishinari, 1984; Watase and Nishinari, 1981, 1982ab, 1984, 1986 and 1988).

Figure II-5 shows the frequency dependence of G' and G" for 1% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. Although the concentration of added $CaCl_2$ was much lower, the viscoelastic behaviour was influenced strongly. Upon addition of 0.85mM $CaCl_2$ (Figure II-5(a)), although the viscoelastic behaviour was similar to that of a 1% solution without salt, both G' and G" at 0℃ were slightly larger. Upon addition of 1.36mM $CaCl_2$ (Figure II-5(b)), G' was larger than those without salt at 30℃ and 0℃, however, at 25℃ or 15℃, G' was too small to be detected at lower frequencies. Upon addition of 1.7mM $CaCl_2$ (Figure II-5(c)), although G' at 30℃ was only slightly larger than those without salt, at 25, 15, or 30℃ the behaviour was strikingly different; G' was larger than G" with little frequency dependence throughout the accessible frequency range. Upon addition of 2mM $CaCl_2$ (Figure II-5(d)), even at 30℃, G' was much greater than G" throughout the experimentally-accessible frequency range, indicating a behaviour of an elastic gel.

Figure II-6 shows the frequency dependence of G' and G" for 2% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. Upon addition of 0.85mM $CaCl_2$ (Figure II-6(a)), at any temperature from 30℃ to 0℃, G' was significantly smaller than those of 2% solutions without salt [Figure II-1(b)]. However, upon addition of 1.7mM $CaCl_2$ [Figure II-6(b)], the behaviour tended towards that of a gel network (weak or elastic gel) at any temperature from 30℃ to 0℃.

Figure II-7 shows the frequency dependence of G' and G" for 1% gellan gum solutions containing $MgCl_2$ of various concentrations at various temperatures. Grasdalen and Smidsrod (1987) have reported that the difference between Ca^{2+} and Mg^{2+} was not so marked for gel strength of gellan gum, however, in the present work, it was found that Ca^{2+} could increase G' more effectively than Mg^{2+}. Upon addition of 0.43mM $MgCl_2$ [Figure II-7(a)], G' was significantly smaller than those without salt at any temperature from 30℃ to 0℃, especially, G' at 30℃ was too small to be detected throughout the experimentally-accessible frequency range. Upon addition of 0.85mM $MgCl_2$, at 15 or 0℃, G' was slightly larger than those without salt. Upon addition of 1.7mM $MgCl_2$, at lower temperature (15℃ or 0℃) G' was significantly larger than those without salt, however G' at 25℃ was too small to be detected at low frequencies and G' at 30℃ was detected throughout the experimentally-accessible frequency range. This result showed a similar tendency to that of a 1% solution with 50mM NaCl (Figure II-1(e)) or 25mM KCl [Figure II-4(d)], and thus, confirmed the conflicting roles of added salt depending on temperatures. Milas et al. (1990) have suggested by using circular dichroism that above the melting temperature T_m, no ionic selectivity existed for the coil conformation of gellan gum, in contrast that below T_m, a strong aggregation existed with ionic selectivity yielding an order of effectiveness in promoting the aggregation $K^+ > Na^+ > Li^+ > TMA^+$. It is, however, found in the present work that even if above the melting temperature, with increasing of ionic strength, the electrostatic repulsion within the coil of gellan gum is gradually screened, so that the coil dimensions collapse, which induced the decrease of G'.

Figure II-8 shows the frequency dependence of G' and G" for 2% gellan gum solutions containing $MgCl_2$ of various concentrations at various temperatures. Upon addition of 0.85mM $MgCl_2$ [Figure II-8(a)], G' was smaller than those without salt at any temperature from 30℃ to 0℃. However, on addition of 1.7mM $MgCl_2$ (Figure II-8(b)), at any temperature, the behaviour tended towards that of a weak gel.

As is seen from Figures II-1 and II-4~8, upon addition of salt of fairly lower concentrations (5mM NaCl(Figure II-1(a)), 0.85mM $CaCl_2$ (Figures II-5(a) and II-6(a)), 0.43mM $MgCl_2$ (Figures II-7(a) and II-8 (a))) except for KCl, G' was significantly smaller than that without salt. Thus, this appears to be related to the fact that K^+ is a structure disordering ion, and in contrast, that Na^+, Ca^{2+} and Mg^{2+} are structure ordering ions in the order of effectiveness : $Na^+ < Ca^{2+} < Mg^{2+}$ (Uedaira, 1990). Here, in the case of the structure ordering ions such as Na^+, Ca^{2+} or Mg^{2+}, if the salt of fairly lower concentration was added, those ions can deprive the hydrated water molecules surrounding carboxyl side groups in gellan gum molecules, and as a result, the repulsion of these "naked" carboxyl groups becomes stronger by the presence of those ions. Therefore, as for the fairly low concentration of those cations, it seems that the effects of highly hydrated ions on the carboxyl side groups in gellan gum molecules are larger than those of screening of the charges on the carboxyl groups. However, the structure disordering ions as K^+ don't deprive the hydrated water molecules surrounding carboxyl side groups, so that the effects of shielding the electrostatic repulsion of carboxyl side groups dominate. Further work, however, is required to elucidate this point.

Temperature dependence

Figure II-9(a)~(f) shows the temperature dependence of G' and G'' during cooling or heating process for a 1.0% gellan gum solution containing NaCl of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5℃/min. Both the storage modulus G' and loss modulus G'' of gellan gum solutions increased remarkably by the addition of NaCl. Upon addition of 10mM NaCl [Figure II-9(a)], G' began to be detected around 29℃ during cooling process and became too small to be detected during heating process around 30℃, while G'' showed a step-like change around 33℃. Upon addition of 20mM NaCl [Figure II-9(b)], G' began to be detected around 30℃ during cooling process and then increased gradually, however, it showed another step-like increase around 10℃, while it decreased rapidly up to 10℃ and then decreased gradually and eventually became too small to be detected around 32℃ during heating process. G'' upon addition of 20mM NaCl showed two step-like changes at both 35℃ and 10℃, indicating two thermal transitions, however G'' showed larger values than G' throughout accessible temperature range. In the case of gellan gum solutions without salt, although G'' for gellan gum solutions whose concentration are lower than 2.0% showed two step-like increase, the higher temperature process corresponded to the helix-coil transition (T_{hc}) and the lower temperature process almost coincided with that of sol-gel transition (T_{sg}). In gellan gum solution containing salts, the temperature at which G'' showed the first step increase in the cooling corresponded to the helix-coil transition (T_{hc}), but the temperature at which it showed the second step increase should not be attributed to that of sol-gel transition. In the previous study, the viscoelastic behaviour for a 1% gellan gum solution in the presence of 20mM NaCl was typical of a dilute solution or concentrated polymer solution at any temperature from 30℃ to 0℃. Thus, it seems that a certain ordered structure was formed by addition of NaCl at the temperature at which G'' showed the second step increase in the cooling process following the helix-coil transition, however, this transition does not produce enough aggregates to reach the gel state. Upon addition of 25mM NaCl [Figure II-9(c)], G'' showed two step-like changes at both 37℃ and 18℃, thus, both transition temperatures shifted significantly to higher temperatures than those in the presence of 20mM NaCl [Figure II-9(b)], moreover, the cross-over of G' and G'' in the presence of 25mM NaCl was observed around 10℃ and then G' became slightly larger than G'' at lower temperatures than the cross-over temperature. This cross-over temperature of G' and G'' may be attributed to the sol-gel transition temperature (T_{sg}). Upon addition of 30mM NaCl (Figure II-9(d)), the temperature at which G'' showed the second step increase in the cooling and the the cross-over temperature (T_{sg}) shifted significantly to higher temperatures than those in the presence of 25mM NaCl [Figure II-9(c)], although T_{hc} was not so different from that in the presence of 25mM NaCl [[Figure II-9(c)]. Upon addition of 40mM NaCl (Figure II-9(e)), G' during cooling process began to be detected around 34℃ and then increased monotonically with decreasing temperature, however, it showed two step-like changes during heating process at both 35℃ and 38℃. G'' in the presence of 40mM NaCl showed two step-like changes at both 38℃ and 32℃ during both cooling and heating process, and the cross-over of both moduli was observed at 17℃. As shown in Figure II-9(b)~(e), the difference between first and second step increase temperatures of G'' decreased and the cross-over temperature of both moduli shifted to higher temperatures with increasing concentration of NaCl. Upon addition of 50mM NaCl (Figure II-9(f)), G' during cooling process began to increase rapidly at 37℃ and then G' became significantly larger than G'', while it decreased monotonically with increasing temperature during heating process, and eventually, it became too small to be detected at 47℃. This indicated that thermal hysteresis was significantly exhibited. G'' in the presence of 50mM NaCl showed one step-like increase at 40℃ in the cooling process, however, G'' showed two step decrease at both 43℃ and 47℃ in the heating process. Moreover, the values of G' and G'' in the presence of 50mM NaCl at lower temperatures were significantly larger than those for 2% or 3% gellan gum solution without salt [Figure II-1(b) and (c)] in Chapter I, which confirmed that the rheological properties of gellan gum solutions were influenced strongly by the presence of sufficient salt.

Figure II-10(a)~(c) shows the temperature dependence of G' and G'' during cooling or heating process for a 1.0% gellan gum solution containing KCl of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5℃/min. Upon addition of 5mM KCl [Figure II-10(a)], G' began to be detected around 27℃ during cooling process and became too small to be detected during heating process around 30℃, while G'' showed a step-like change around 32 ℃. The viscoelastic behaviour was typical of a dilute polymer solution at any temperature from 30℃ to 0℃ for a 1% gellan gum solution containing 5mM KCl as shown in in the frequency dependence of both moduli for a 1% gellan gum solution containing 5mM KCl [Figure II-4(a)]. Upon addition of 10mM KCl [Figure II-10(b)], G' began to be detected around 30℃ during cooling process and then increased gradually, moreover, the cross-over of G' and G'' was observed around 8℃ and then G' became slightly larger than G'' at lower temperatures than the cross-over temperature. G'' upon addition of 10mM KCl [Figure II-10(b)] showed two step-like changes at both 33℃ and 22℃, indicating two thermal transitions, however, the lower transition temperature at which G'' drastically changed was significantly higher than the cross-over temperature of both moduli. In the case of gellan gum solutions without salt, although G'' for gellan gum solutions whose concentrations are lower than 2.0% showed two step-like increase, the higher temperature process corresponded to the helix-coil transition (T_{hc}) and the lower temperature process almost coincided with that of sol-gel transition (T_{sg}). However, the temperature at which G'' showed the first step increase in the cooling corresponded to the helix-coil transition (T_{hc})

for gellan gum solution containing salts, however, the temperature at which it showed the second step increase should not be attributed to that of sol-gel transition. As shown in the frequency dependence of both moduli for a 1% gellan gum solution in the presence of 10mM KCl [Figure II-4(b)], the viscoelastic behaviour was typical of a dilute solution at 30 or 25℃ and was typical of a concentrated polymer solution at 15℃, however the behaviour at 0℃ changed to that of a weak gel. Thus, it seems that a certain ordered structure was formed by the addition of KCl at the temperature at which G'' showed the second step increase in the cooling process following the helix-coil transition, however, this transition does not produce enough aggregates to reach the gel state. Therefore, the cross-over temperature of G' and G' may be attributed to the sol-gel transition temperature (T_{sg}). It was found that the difference between first and second step increase temperature of G'' decreased and the cross-over temperature of both moduli shifted to higher temperatures with increasing concentration of KCl. Upon addition of 30mM KCl [Figure II-10(c)], G' during cooling process began to be detected at 37℃ and then G' became significantly larger than G'', while it decreased monotonically with increasing temperature during heating process, and eventually, it became too small to be detected at 43℃. This indicated that thermal hysteresis was significantly exhibited. G'' in the presence of 30mM KCl showed one step-like increase at 40℃ in the cooling process, however, G'' showed two step decrease at both 40℃ and 43℃ in the heating process. In the case of the frequency dependence of both moduli for a 1% gellan gum solution containing 30mM KCl [Figure II-4(e)], the viscoelastic behaviour tended towards that of a weak gel even at a relatively higher temperature such as 30℃. Moreover, the values of G' and G'' in the presence of 30mM KCl at lower temperatures were significantly larger than those for 2% or 3% gellan gum solution without salt [Figure II-1(b) and (c) in Chapter I], which confirmed that the rheological properties of gellan gum solutions were influenced strongly by the presence of sufficient salt. The effect of NaCl on viscoelasticity of gellan gum solutions was almost similar to that of KCl, however KCl influences the viscoelastic behaviour of gellan gum solutions more effectively than NaCl. This reason is interpreted on the basis that the cations such as Na^+ are the structure ordering ions for water, and the cations such as K^+ belong to the structure disordering ions (Suzuki, 1980; Watase and Nishinari, 1981, 1982ab, 1984, 1986; Uedaira and Ohsaka, 1990) as described above.

Figure II-11(a)~(c) shows the temperature dependence of G' and G" during cooling or heating process for a 1.0% gellan gum solution containing $CaCl_2$ of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5℃/min. The viscoelastic behaviour of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. Upon addition of 0.85mM $CaCl_2$ [Figure II-11(a)], G' began to be detected around 22℃ during cooling process and became too small to be detected during heating process around 28℃, while G" showed a step-like change around 30℃. In the frequency dependence of both moduli for this solution [Figure II-6(a)], the viscoelastic behaviour was typical of a dilute solution at any temperature from 30℃ to 0℃. Upon addition of 1.7mM $CaCl_2$ [Figure II-11(b)], G' during cooling process began to be detected at 29℃ and then G' became significantly larger than G", while it decreased monotonically with increasing temperature during heating process, and eventually, it became too small to be detected at 41℃. G" in the presence of 1.7mM $CaCl_2$ showed a step-like change at 33℃, however the difference between G" in the cooling and heating process at higher temperature sides was markedly observed, which indicated that thermal hysteresis was significantly exhibited in this solution. In the frequency dependence of both moduli for this solution [Figure II-6(c)], the viscoelastic behaviour remained typical of a dilute solution at 30℃, however, the behaviour became typical of a weak gel at any temperature lower than 30℃, which corresponded well with that in the temperature dependence of both moduli. Upon addition of 2.04mM $CaCl_2$ [Figure II-11(c)], G" increased rapidly and G' began to be detected at 33℃ in the cooling process, however, there was no evidence for remarkable change of both moduli up to 60℃. In the case of added sufficient monovalent cations [Figure II-9(f) and Figure II-10(c)], although the temperature at which G' and G" increased rapidly was significantly higher than that in the presence of 2.04mM $CaCl_2$, these junction zones formed in the presence of sufficient monovalent cations were completely melted on heating to 50℃. Therefore, the thermal behaviour in the presence of divalent cations was obviously different from that without salts or in the presence of monovalent cations, as illustrated in the DSC measurement described later. In the case of the frequency dependence of both moduli upon addition of 2.04mM $CaCl_2$ [Figure II-6(d)], the viscoelastic behaviour tended towards that of an elastic gel even at a relatively higher temperature such as 30℃.

Figure II-12(a)~(d) shows the temperature dependence of G' and G'' during cooling or heating process for a 0.8% gellan gum solution containing $MgCl_2$ of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5℃ /min. The viscoelastic behaviour of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. G' for 0.8% gellan gum solution without salt [Figure II-12(a)] was too small to be detected in both cooling and heating process, and upon addition of 0.85mM $MgCl_2$ [Figure II-12(b)], it was also too small to be detected in both cooling and heating process, while both solutions showed one step like change corresponding to the helix-coil transition (T_{hc}). In the presence of 0.85mM $MgCl_2$, however, T_{hc} shifted to higher temperatures and the relaxational strength at T_{hc} increased slightly compared to that without salt. Upon addition of 1.7mM $MgCl_2$, G' began to be detected at 27℃ in the cooling process and became too small to be detected at 38℃ in the heating process. G'' in the presence of 1.7mM $MgCl_2$ [Figure II-12(c)] showed a step-like change at 30℃, however the significant difference between G'' in the cooling and heating process at higher temperature sides was significantly observed. Temperature dependence of both moduli in the presence of 2.04mM $MgCl_2$ (Figure II-12(d)) was markedly different; G'' increased rapidly and G′ began to be detected at 31℃ in the cooling process, however, there was no evidence for remarkable change of both moduli up to 60℃. Upon addition of sufficient monovalent cations [Figure II-10(f)], although the temperature at which G' and G'' increased rapidly was significantly higher than that in the presence of 2.04mM $MgCl_2$, these junction zones formed in the presence of sufficient monovalent cations were completely melted on heating to 50℃. Therefore, the thermal behaviour in the presence of divalent cations was obviously different from that without salts or in the presence of monovalent cations, as illustrated in the DSC measurement. As shown in Figure II-11, the effect of $CaCl_2$ on the viscoelasticity of gellan gum solutions was almost similar to that of $MgCl_2$, however $CaCl_2$ can influence the viscoelastic behaviour of gellan gum solutions more effectively than $MgCl_2$. The carboxyl side groups in gellan gum molecules repulse each other by electrostatic interaction, and this hinders the tight binding of helices and also the tight aggregation of helices (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986; Crescenzi et al., 1988; Dentini et al., 1988; Chapman et al., 1990; Milas, et al., 1990; Robinson et al., 1991; Manning, 1992; Shi, 1990), so that the introduction of cations can shield the electrostatic repulsion and thereby permits tight binding and aggregation of helices at lower temperatures or leads to the reduction of coil dimensions at higher temperatures (Robinson et al., 1991; Kobayashi et al., 1994). It was suggested that the influence of cations on the viscoelastic behaviour of gellan gum solutions was more remarkable for K^+ than Na^+, and for Ca^{2+} than Mg^{2+} as in the previous study (Miyoshi et al., 1994a) and divalent cations were more effective than monovalent cations.

DSC

Figures II-13~16 show cooling and heating DSC curves of 1% gellan gum solutions with and without NaCl or KCl of various concentrations. Cooling or heating rate was chosen as 0.5℃/min in all the measurements. The cooling curve for a 1% gellan gum solution without salt (0mM) showed a single exothermic peak at 29.0℃, and the heating curve showed a single endothermic peak at 30.5℃. DSC cooling curves showed a single exothermic peak in all the cases, with T_s shifting to progressively higher temperatures with increasing concentration of the added NaCl or KCl [Figures II-13(a), II-14(a), II-15(a) and II-16(a)], corresponding well with rheological results; storage modulus G' increased by the addition of NaCl up to 20mM or KCl up to 15mM. As discussed in rheological measurement, these results were caused by the shielding of electrostatic repulsion between carboxyl groups in the gellan gum molecules, because the alkali metal ions shield the electrostatic repulsion of the carboxyl groups which prevent the tight binding and the aggregation of the helices (Manning, 1992; Shi, 1990; Crescenzi and Smidsrod, 1987; Crescenzi et al., 1986 and 1988, Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991). Although T_s shifted to higher temperatures with increasing concentration of added NaCl or KCl, G' as a function of the concentration of added NaCl or KCl showed a complicated behaviour as shown in the rheological measurement. The exothermic enthalpy ΔH as a function of the concentration of added NaCl or KCl also showed a complicated behaviour [Figure II-14(a) and Figure II-16(a)] which is similar to that of G'. The addition of salts seems to increase the thermal stability of junction zones, and the number of junction zones up to a certain concentration of salt. However, above this concentration of salt, the number of junction zones or the number of elastically active chains seems to decrease because the observed G' decreased (Figures II-1 and II-5). Since the addition of excessive salt seems to form junction zones of different structures as described below, the situation is not simple and cannot be explained by a simple mechanism.

At low concentrations of NaCl or KCl, the DSC heating curves[Figures II-13(b) and II-15(b)] also showed a single endothermic peak. With progressive addition of NaCl or KCl, however, the melting endothermic peak gradually developed bimodal character and eventually splitted into more than two peaks [Figures II-14(b) and II-16(b)]. These results were in good agreement with those obtained by Manning (1992), and the multiple peaks of gellan gum solutions observed by Moritaka et al. (1992) appear to involve the effect of metal ions in the samples. In the presence of sufficient salt, junction zones of different structures especially with higher bonding energy or lower rotational freedom may be formed as mentioned above, and as a result, higher temperature peaks appear at the expense of the exothermic enthalpy ΔH for a main exothermic peak. However, some junction zones don't melt below 90℃ as will be discussed later. The value of ΔH for a main peak around 30℃~45℃ in the cooling DSC increased with increasing concentration of NaCl or KCl, however, ΔH with ca. 100mM NaCl was smaller than that with 50mM or 75mM NaCl (Figures II-13(a), II-14(a), II-15(a) and II-16(a)). A gellan solution containing KCl showed a higher T_s or T_m than a gellan solution containing NaCl of the same concentration. This was in agreement with the results of viscoelastic measurements (Figures IIand II-4), and could be explained by the difference of these two ions : K^+ is the structure disordering ion whilst Na^+ is the structure ordering ion (Uedaira, 1990). Moreover, the onset of detectable splitting occurred at a salt concentration of about 60mM NaCl or 50mM KCl and coincides with the onset of elastic gel-formation ; it was found from viscoelastic measurements (Figures II-1 and II-4) that an elastic gel network was formed even at a higher temperature as 30℃ in the presence of sufficient salt such as 60mM NaCl or 50mM KCl. In other words, on addition of sufficient salt to form elastic gels [e.g.75mM NaCl(Figure II-1(f)) or 50mM KCl(Figure II-4(f)], the gellan gum solutions seemed to show multiple thermal transitions; heating DSC curves of a 1% gellan gum solution containing 75mM NaCl or 50mM KCl showed more than two endothermic peaks. In terms of zipper model approach (Nishinari et al., 1990), the appearance of two or more endothermic peaks in heating DSC curves for gellan gum solutions in the presence of sufficient salt suggests that the zippers with different bonding energies or different degree of rotational freedom are formed in the presence of sufficient metal ions. The lower temperature endothermic peak is attributed to the melting of zippers with lower bonding energies or with higher rotational freedoms whilst the higher temperature endothermic peak corresponds to the melting of zippers with higher bonding energies or with lower rotational freedoms. Gellan gum solutions in the presence of sufficient monovalent cations (100mM KCl) showed a single exothermic peak on cooling at about 45℃ [Figure II-16(a)], and a single endothermic peak on heating at about 80℃ [Figure II-16(b)]; this thermal hysteresis was far more pronounced with comparison to that observed in gellan gum gels with less salts. Therefore, it was considered that with increasing concentration of the added salt, the formation of aggregated helical structures was promoted, and eventually the higher temperature peak originating from thermally-stable junction zones of the gel network was observed.

Figures II-17 and II-18 show cooling and heating DSC curves of 2% gellan gum solutions containing NaCl or KCl of various concentrations. The 2% gellan gum solutions containing NaCl or KCl showed a similar tendency to that of 1% gellan gum solutions with the same concentration of salt (Figures II-13 and II-16), however, heating DSC curves with more than 50mM NaCl (Figure II-17) or 25mM KCl (Figure II-18) which is lower salt concentration than in the case of 1% gellan gum solutions [75mM NaCl (Figure II-14) or 50mM KCl(Figure II-16)], splitted into more than two peaks. This suggested that even if the concentration of monovalent cations added was the same, the helix aggregation in gellan gum was more promoted as polymer concentration was increased as suggested by Manning (1992) and Nakamura et al. (1993).

Figures II-19 and II-20 show cooling and heating DSC curves of 1% gellan gum solutions containing $CaCl_2$ or $MgCl_2$ of various concentrations. The behaviour of 1% gellan gum solutions with divalent cations was different from that with monovalent cations (Figures II-13~II-16). Although T_s shifted to higher temperatures with increasing concentration of added $CaCl_2$ or $MgCl_2$, the exothermic enthalpy estimated for a main peak at 30~33℃ increased up to a concentration of 0.85mM $CaCl_2$ or $MgCl_2$ and then decreased, however many other peaks were observed at higher temperatures with increasing concentration of salt [Figures II-19(a) and II-20(a)]. On the other hand, T_m shifted to higher temperatures up to a concentration of 1.7mM $CaCl_2$ or $MgCl_2$ and then shifted to lower temperatures with increasing concentration of salt [Figures II-19(b) and II-20(b)]. However, the endothermic enthalpy estimated for a main peak increased up to a concentration 0.85mM $CaCl_2$ or $MgCl_2$ and then decreased with increasing concentration of salt, although many other peaks were observed, especially at higher temperatures [Figures II-19(b) and II-20(b)]. Moreover, the endothermic peaks for gels containing over 3.4mM $CaCl_2$ or $MgCl_2$ were too broad to be resolved from the base line [Figures II-19(b) and II-20(b)], however, in contrast the exothermic peaks were much sharper and readily recognized [Figures II-8(a) and II-20(a)].

Figures II-21 and II-22 show cooling and heating DSC curves of 2% gellan gum solutions containing $CaCl_2$ or $MgCl_2$ of various concentrations. The 2% gellan solutions containing $CaCl_2$ or $MgCl_2$ of various concentrations showed a similar tendency to that of 1% gellan solutions with the same concentration of salt (Figures II-19 and II-20), however, with addition of higher concentration of salt (5.1mM) [Figures II-21(b) and II-22(b)] than in the case of 1% gellan gum solutions (3.4mM) [Figures II-19(b) and II-20(b)], endothermic peaks in heating DSC curves were too broad to be resolved from the baseline. Moreover, in cooling DSC curves for 2% solutions compared to 1% solutions, when the concentration of added $CaCl_2$ or $MgCl_2$ was in excess (above 3.4mM) , a second exothermic peak began to develop at a higher temperature and shifted to higher temperatures with increasing concentration of the added salt [Figures II-21(a) and II-22(a)].

Since the electric charges of divalent cations such as Ca^{2+} or Mg^{2+} are larger than those of monovalent cations as Na^+ or K^+, divalent cations can shield the electrostatic repulsion of the carboxyl groups more effectively than can monovalent cations, as in the case of κ-carrageenan gels where the electrostatic repulsion of sulfate groups is shielded more effectively by divalent cations than by monovalent cations (Watase and Nishinari, 1986). However, it is also considered that divalent cations may make cross-links by ionic bonds, so that those can form thermo-irreversible gels involving specific cation- polyanion interaction in addition to ion-specific charge screening effects (Manning, 1992; Watase and Nishinari, 1986; Tsutsumi et al., 1993). It is, moreover, suggested in the present work that the network of gellan gum gels containing $CaCl_2$ or $MgCl_2$ is formed by various junction zones. This effect results in the formation of more heterogeneous systems containing thermally-stable junction zones. However, it is not necessarily true that those junction zones involving the specific cation-polyanion interaction cannot be melt above 100℃, as shown below.

Figures II-23~II-26 show cooling and heating DSC curves in several cycles for a 1% gellan gum solution with 6.8mM $CaCl_2$, 6.8mM $MgCl_2$ 100mM NaCl or 100mM KCl. These experiments were carried out on the sample with sufficient divalent cations which showed the broad exothermic peaks which cannot be resolved from baseline, or the sample with sufficient monovalent cations which showed multiple endothermic peaks in DSC measurements. In the presence of sufficient divalent cations (Figure II-23 or II-24), the T_S around 34.2℃ in the second scan from 110℃ was in good agreement with that in the first scan, which indicates that the various junction zones in the presence of sufficient divalent cations were almost unzipped on heating to 110℃. However, in the case of cooling DSC curves from 100, 90, 80 70℃, T_S significantly shifted to lower temperatures and the exothermic enthalpies estimated for a main peak around 30℃ decreased with decreasing temperature of the initial heating, and eventually in cooling DSC curve from 70℃, no main peak was observed around 30℃ and many other peaks were observed at both high and low temperatures. Thus, gellan gum solutions with sufficient divalent cations form firm gels on cooling to below the setting temperature, and become more heat resistant. However, the possibility of the formation of ionic bonds by divalent cations in gellan gum molecules may be excluded because gellan gum gels in the presence of divalent ions almost melted on heating up to 110℃ as described above. The bond energy for ionic bonds or covalent bonds is far higher than that for hydrogen bonds or for hydrophobic interactions. These DSC results were in good agreement with the rheological results shown in Figures II-23 and II-24. If gellan gum gels in the presence of sufficient divalent cations could be heated to 110℃ in the viscoelastic measurements, these elastic gel network might be melted, so that G' might begin to decrease rapidly although this was unfortunately not possible to execute experimentally.

In the presence of 100mM NaCl (Figure II-25), the T_S at 42℃ in the second scan from 110℃ was in good agreement with that in the first scan. T_S of 1% gellan gum solution in the presence of 100mM NaCl was significantly higher than that in the presence of 6.8mM $CaCl_2$ or $MgCl_2$, however, the cooling DSC curve from 100℃ or 80℃ was also in close agreement with that from 110℃, which indicates that the ordered structures formed by the thermally-stable junction zones were almost unzipped on heating to 80℃. Thus, this indicates that these helices were almost melted on heating to 80℃. It is explained by the fact that the endothermic peaks were observed at temperatures lower than 80℃. However, in case of cooling DSC curves from 60℃, T_S significantly shifted to lower temperatures and the exothermic enthalpy decreased markedly compared to those in the curves from 110℃ or 80℃. In the presence of sufficient KCl (Figure II-26), the behaviour was similar to that in the presence of sufficient NaCl. Thus, T_S at 46.9℃ in the second scan from 110 ℃ was in good agreement with that in the first scan, and the cooling DSC curve from 100℃ or 90℃ was also in close agreement with that from 110℃, which indicates that the ordered structures formed by the thermally-stable junction zones were almost unzipped on heating to 90℃. In the cooling DSC curve from 80℃, T_S significantly shifted to lower temperatures and the exothermic enthalpy decreased markedly compared to those in the cooling curve from 90℃, 100℃ or 110℃. These DSC results in the presence of sufficient monovalent cations were also in good agreement with the rheological results. Therefore, the mechanism of gel formation in gellan gum with divalent cations is markedly different from that with monovalent cations.

Figure II-27 shows cooling and heating DSC curves of a 3.2% gellan gum solution without salt and of a 1% gellan gum solution with 75mM NaCl and cooling or heating rate of 0.5℃/min. As shown in Figure II-13~II-18, in the presence of small amount of monovalent cations, the DSC heating curves showed a single endothermic peak, however, on further addition of salt, they gradually developed bimodal character and eventually splitted into multiple peaks, although the DSC cooling curves showed a single exothermic peak in all the cases. In the absence of salts, the gellan gum solutions whose concentration was higher than 3.2% showed two endothermic peaks in the heating DSC curve, therefore, it was suggested that the gellan gum solutions of high concentrations formed the ordered structures involving thermally-stable junction zones as in the case of added salt. However, as shown in this figure, it is clearly demonstrated that these multiple endothermic peaks for a 1% gellan gum solution in the presence of sufficient monovalent cations were observed at significantly higher temperatures than those for gellan gum solution of a high concentration (3.2%) without salt. Moreover, the exothermic and endothermic enthalpies for a 1% solution with 75mM NaCl were estimated as 13.4 and 21.8J per one gram of gellan gum, while those for a 3.2% gellan gum solution without salt were estimated as 12.4 and 11.9J per one gram of gellan gum. Although the aggregation of gellan gum molecules was promoted with increasing concentration of both polymer and added salt, in the absence of salts, the carboxyl side groups in gellan gum molecules repulse each other by electrostatic interaction, and this hinders the tight binding of helices and also the tight aggregation of helices. However, the presence of cations can shield the electrostatic repulsion and thereby permits tight binding and aggregation of helices.

The exothermic enthalpies for potassium type gellan gum reported by Moritaka et al. were significantly lower than those of our results. This seems to be due to the effects of salts in samples because a main peak was not so clear. In other words, other junction zones with different thermal stabilities may be formed by effects of salts in samples. It has been reported in the case of κ-carrageenan solutions that the exothermic and endothermic enthalpies were estimated as ca. 25~45J per one gram of κ-carrageenan (Snoeren and Payens; 1976; Rochas and Rinaudo, 1982; Norton et al., 1983; Gekko, et al, 1987; Nishinari et al., 1995). Thus, these enthalpies for gellan gum estimated in this work were significantly smaller than those for κ-carrageenan.

In the presence of divalent cations, T_s shifted to higher temperatures with increasing concentration of $CaCl_2$, and the exothermic and endothermic enthalpies estimated for a main peak increased up to a certain concentration of salt and then decreased. However, in addition to a main peak, many other peaks were observed, especially at higher temperatures, with increasing concentration of salt. Moreover, the endothermic peaks in the heating DSC curve for gellan gum solutions in the presence of sufficient divalent cations were too broad to be resolved from baseline, however, in contrast, the main exothermic peak in the cooling DSC curve was much sharper and readily recognized. As described in Figures II-23 and II-24, gellan gum solutions with sufficient divalent cations form firm gels on cooling to below the setting temperature, and these gels became more heat resistant,i.e. a heating DSC curve did not show a clear endothermic peak. This behaviour was very different from that of thermoreversible gels formed by gellan gum alone or in the presence of monovalent cations. Hence, although the cooling DSC curve in the presence of sufficient divalent cations showed a main exothermic peak around 35℃, the various junction zones did not seem to be unzipped completely on heating below 100℃. In this experiment, DSC curves were obtained by cooling and heating samples between 120℃ and 5℃ (Figure II-28). For a 1% gellan gum solution in the presence of 6.8mM $CaCl_2$, many small peaks were observed especially at higher temperatures in the heating DSC curve and the largest endothermic peak was observed at 105.2℃. Therefore, although both T_s and T_m shifted to higher temperatures with increasing concentration of divalent cations, it seemed that in the presence of sufficient divalent cations, the main endothermic peak shifted to a temperature higher than 100℃. It was, moreover, suggegted that the network of gellan gum gels containing divalent cations was formed by various junction zones with different thermal stabilities. However, although divalent cations promote the formation of thermally stable junction zones much more strongly than monovalent cations, the gellan gum gels containing divalent cations may also consist of the physical junction zones by hydrogen bonds because these junction zones can be almost unzipped on heating to 120℃. As mentioned above, some workers (Manning, 1992; Tsutsumi et al., 1993) have suggested that divalent cations may make cross-links by ionic bonds, so that those can form thermo-irreversible gels involving specific cation-polyanion interaction in addition to ion-specific charge screening effects. However, the possibility of the formation of ionic bonds by divalent cations in gellan gum molecules may be excluded because gellan gum gels in the presence of divalent ions melted completely on heating up to 110℃ as mentioned above. The bond energy for ionic bonds or covalent bonds is far higher than that for hydrogen bonds or for hydrophobic interactions. However, more detailed studies based on other methods to describe the phenomena at the molecular level are required.

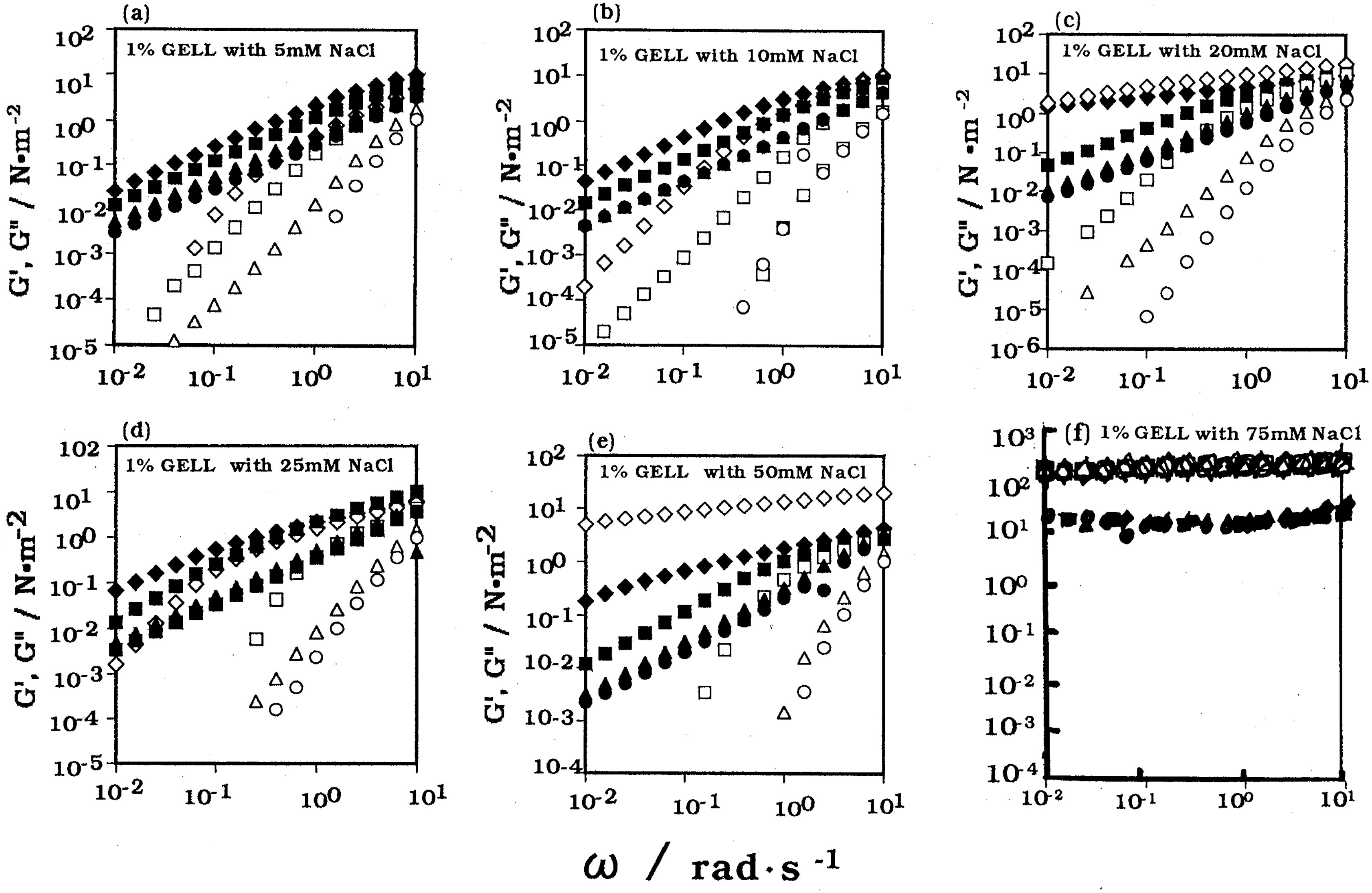

Fig. II-1 Frequency dependence of storage modulus G' and loss modulus G" for 1% gellan gum solutions containing NaCl of various concentrations at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G", 25℃; (□)G',(■)G", 15℃; (◇)G',(◆)G", 0℃. NaCl conc ; 5mM(a), 10mM(b), 20mM(c), 25mM(d), 50mM(e) or 75mM(f),

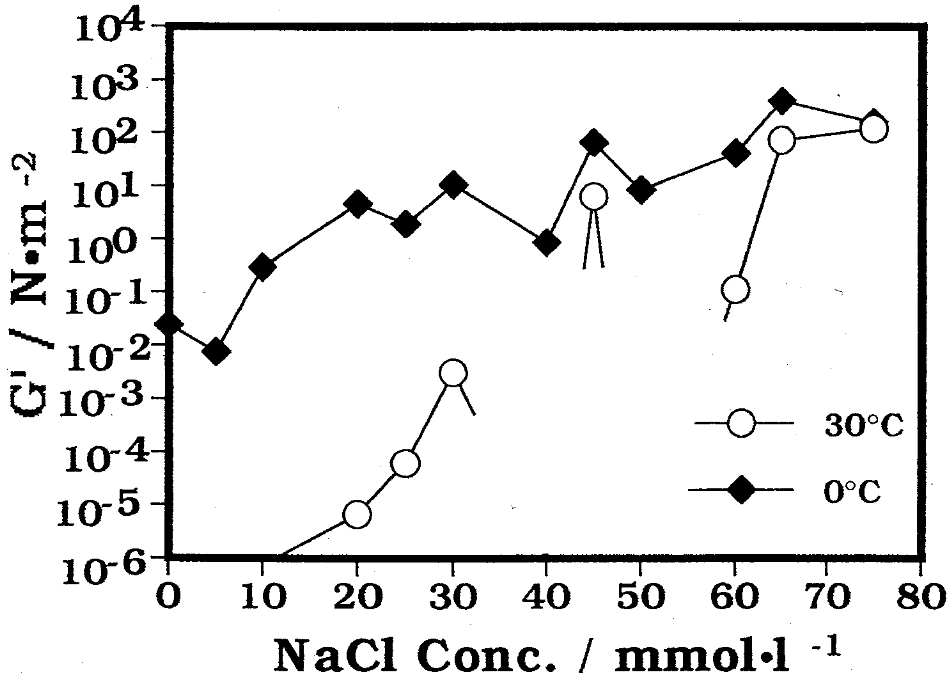

Fig. II-2 Dependence of storage modulus G' for 1% gellan gum solutions at 30℃(○) and 0℃(◇) on the concentration of NaCl. freq.ω =0.1rad/sec.

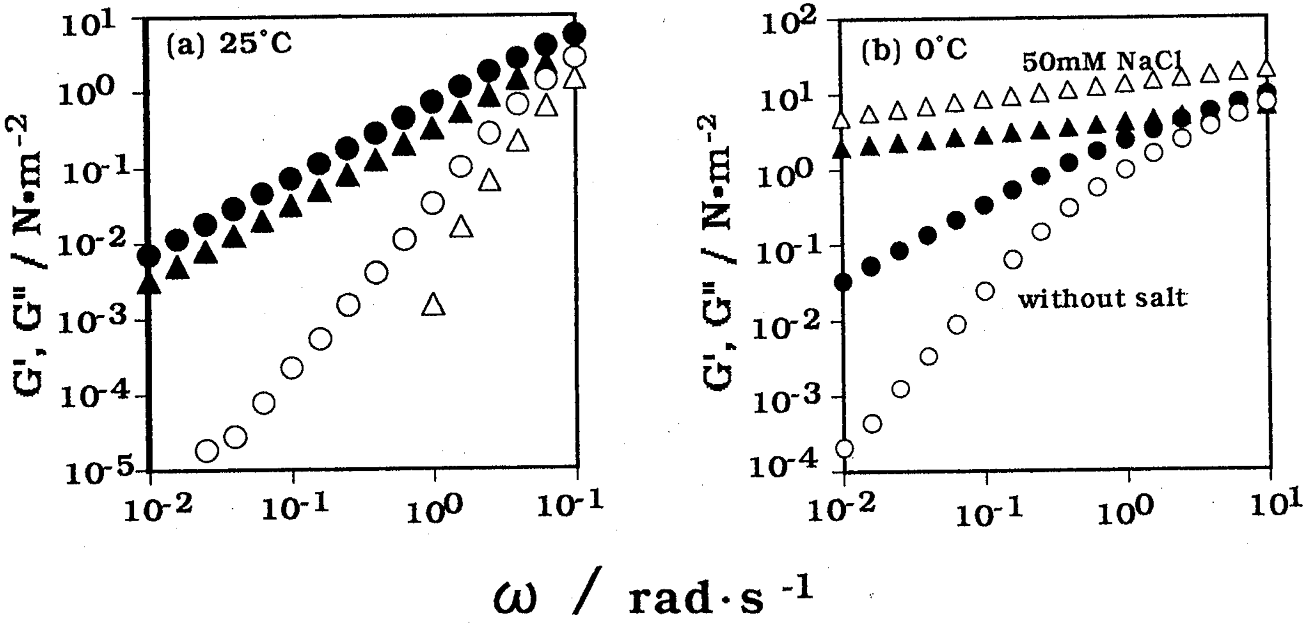

Fig. II-3 Frequency dependence of storage modulus G' (○, △) and loss modulus G"(●, ▲) for 1% gellan gum solutions with and without 50mM NaCl. NaCl conc. ; 0mM (○, ●),50mM (△, ▲), Temperature ; 25℃(a), 0℃(b).

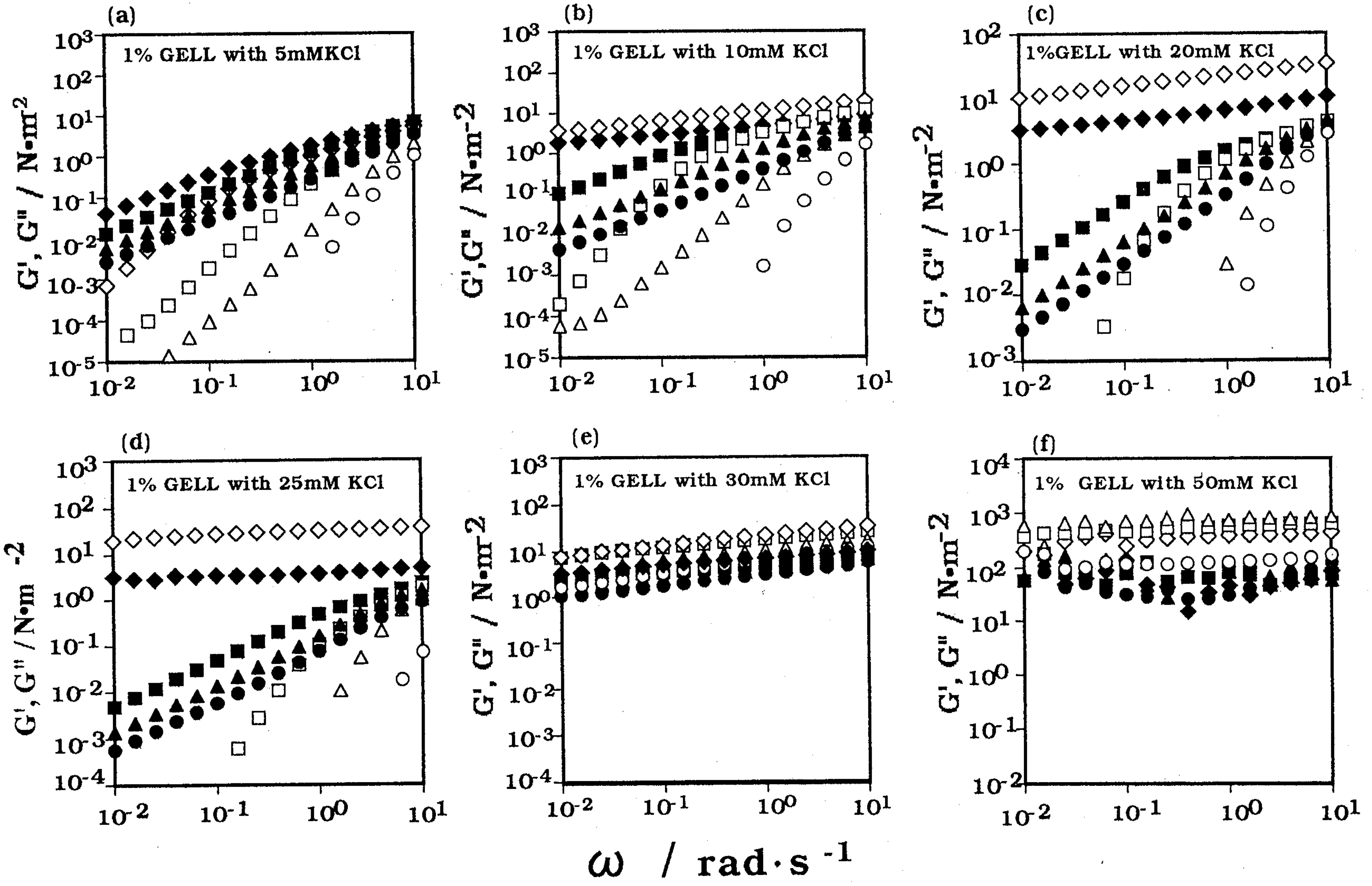

Fig. II-4 Frequency dependence of storage modulus G' and loss modulus G" for 1% gellan gum solutions containing KCl of various concentrations at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G", 25℃; (□)G',(■)G", 15℃; (◇)G',(◆)G", 0℃. KCl conc ; 5mM(a), 10mM(b), 20mM(c), 25mM(d), 30mM(e) or 50mM(f),

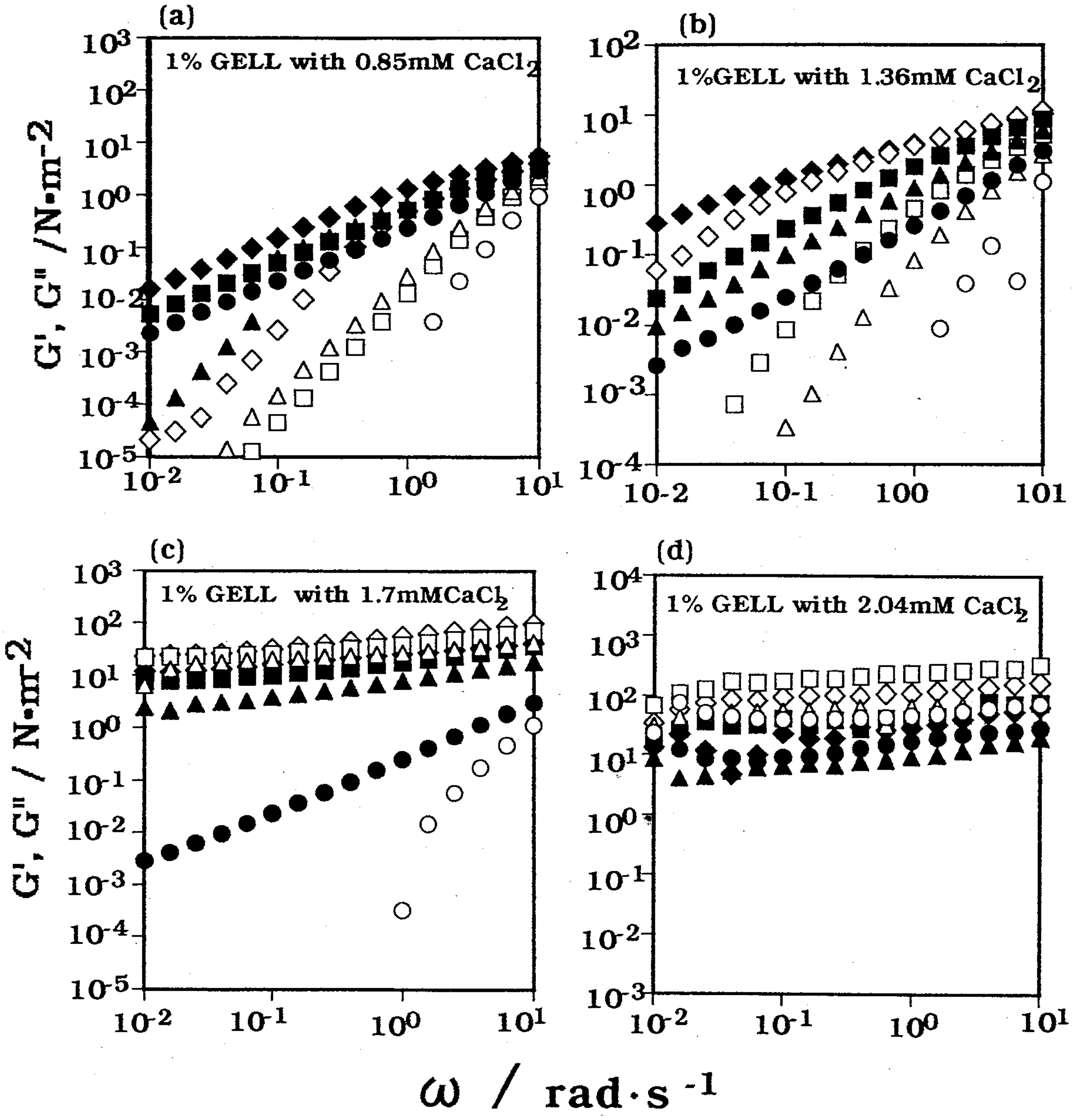

Fig. II-5 Frequency dependence of storage modulus G' and loss modulus G" for 1% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G", 25℃; (□)G',(■)G", 15℃; (◇)G',(◆)G", 0℃. $CaCl_2$ conc ; 0.85mM(a), 1.36mM(b), 1.7mM(c) or 2.0mM(d).

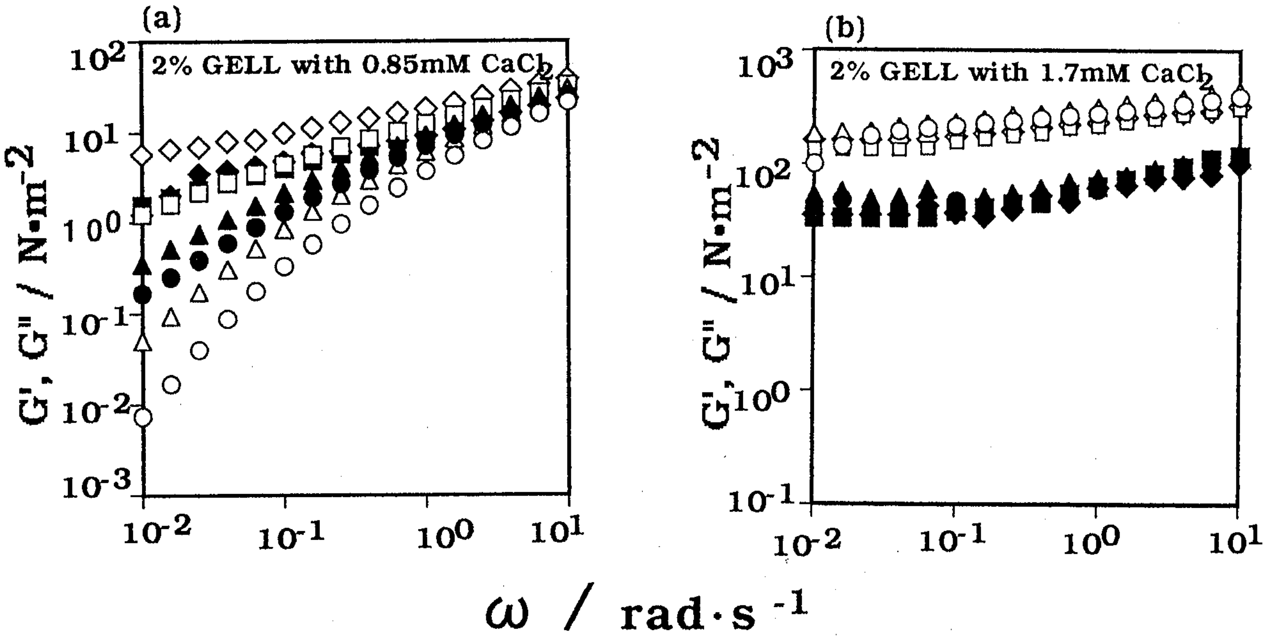

Fig. II-6 Frequency dependence of storage modulus G' and loss modulus G" for 2% gellan gum solutions containing $CaCl_2$ of various concentrations at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G", 25℃; (□)G',(■)G", 15℃; (◇)G',(◆)G", 0℃. $CaCl_2$ conc ; 0.85mM(a) or 1.7mM(b),

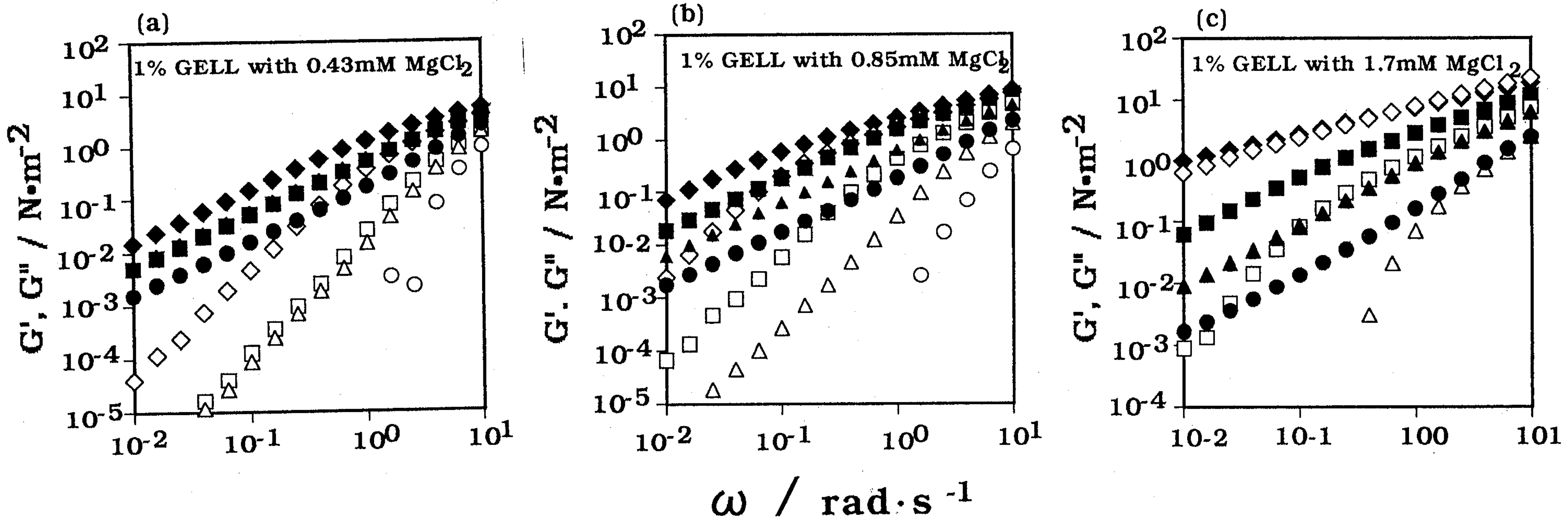

Fig. II-7 Frequency dependence of storage modulus G' and loss modulus G" for 1% gellan gum solutions containing $MgCl_2$ of various concentrations at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G", 25℃; (□)G',(■)G", 15℃; (◇)G',(◆)G", 0℃. $MgCl_2$ conc ; 0.43mM(a) or 0.85mM(b) or 1.7mM(c) or 2.0mM(d),

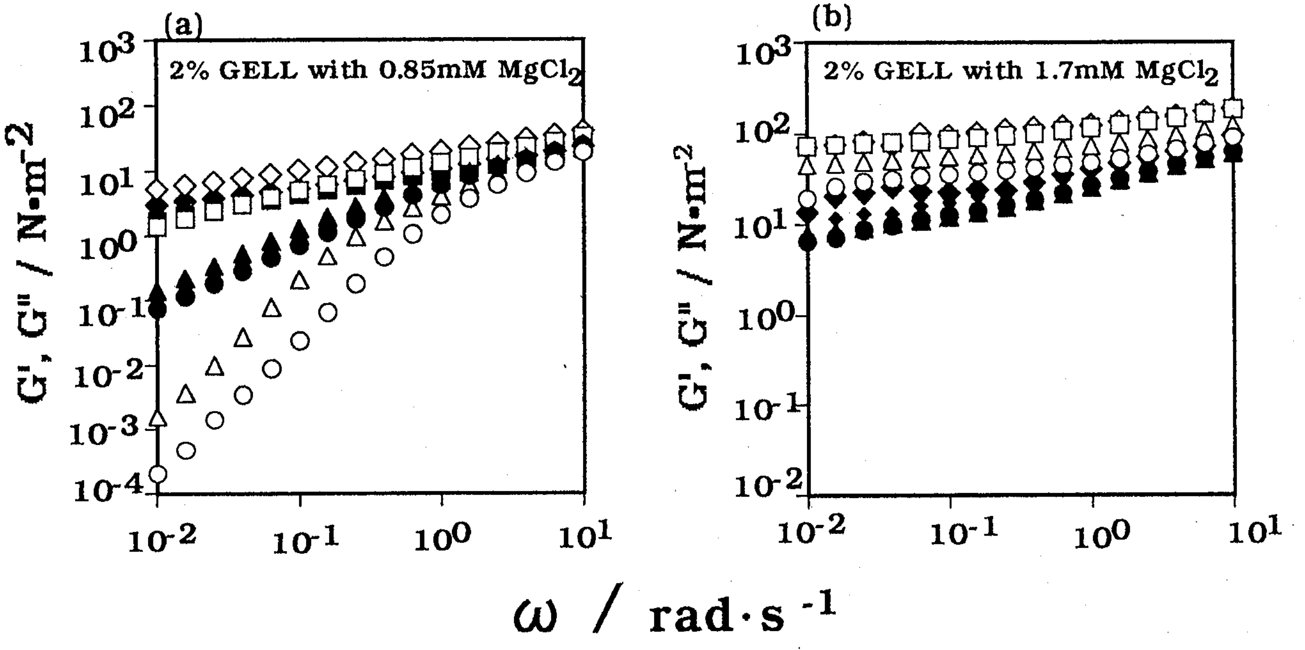

Fig. II-8 Frequency dependence of storage modulus G' and loss modulus G" for 2% gellan gum solutions containing $MgCl_2$ of various concentrations at various temperatures. (○)G', (●)G", 30℃; (△)G', (▲)G", 25℃; (□)G', (■)G", 15℃; (◇)G', (◆)G", 0℃. $MgCl_2$ conc ; 0.85mM(a) or 1.7mM(b).

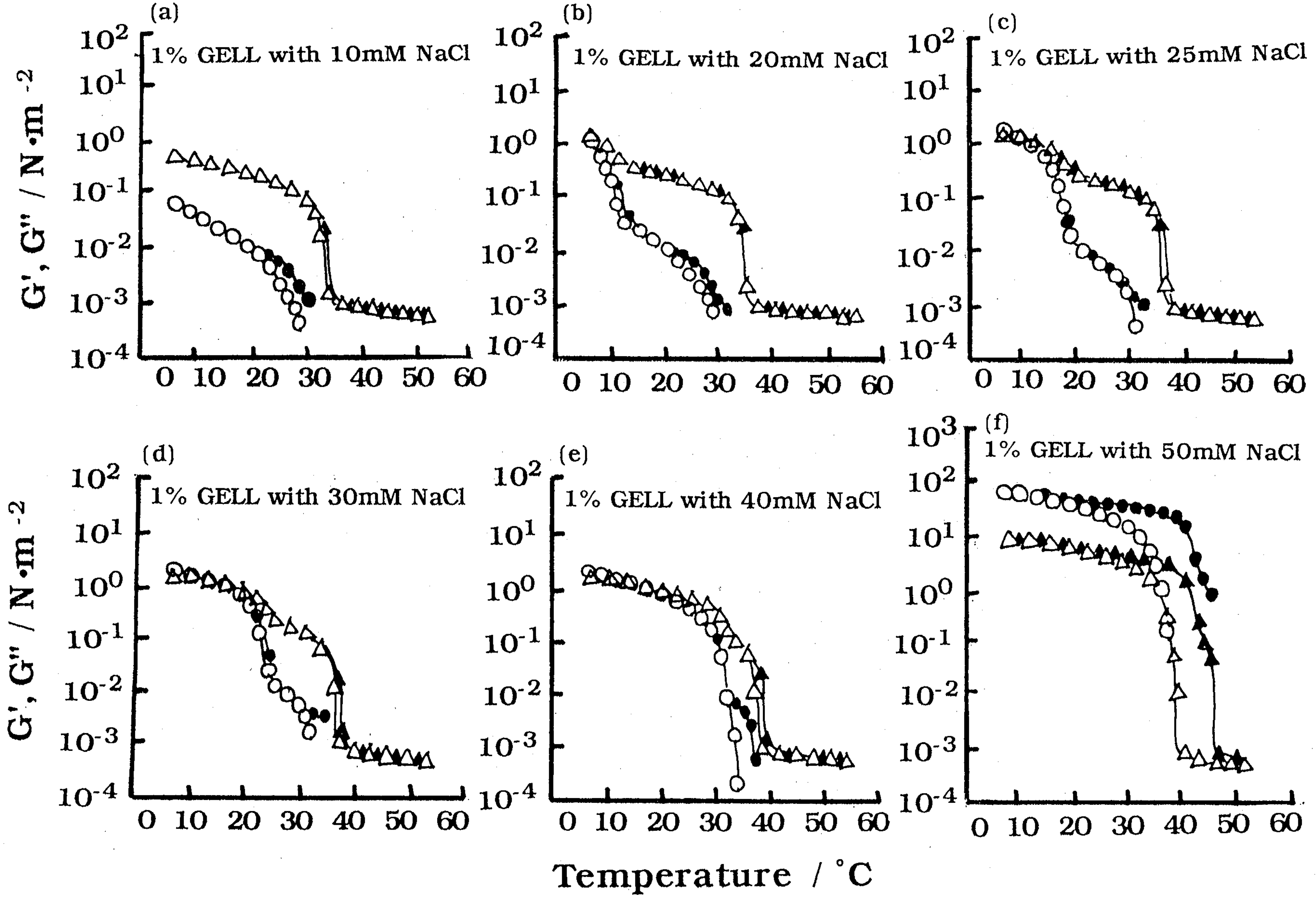

Fig. II-9 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 1.0% gellan gum solution containing NaCl of various concentrations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s;

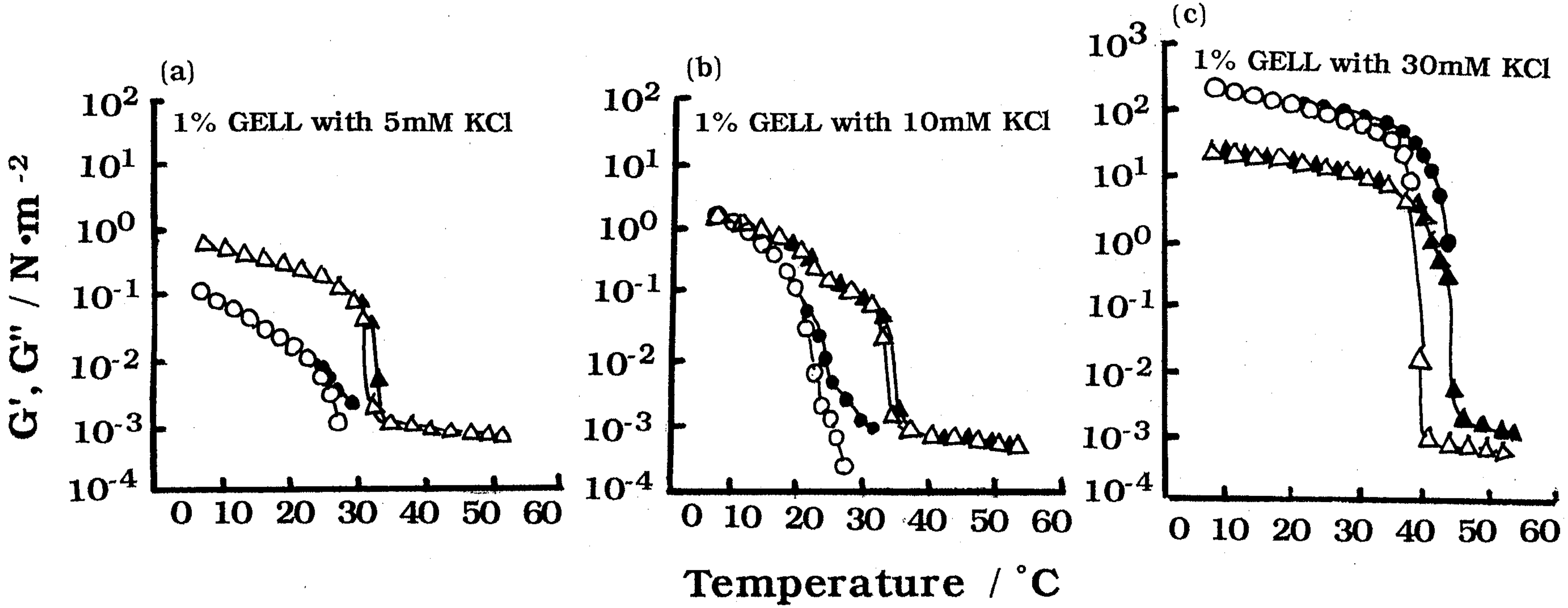

Fig. II-10 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 1.0% gellan gum solution containing KCl of various concentrations. (○)G',(△)G", cooling; (●)G',(▲)", heating; cooling and heating rate, 0.5℃/min; freq.ω=0.1rad/s. KCl conc.: (a), 5mM; (b), 10mM; (c), 30mM.

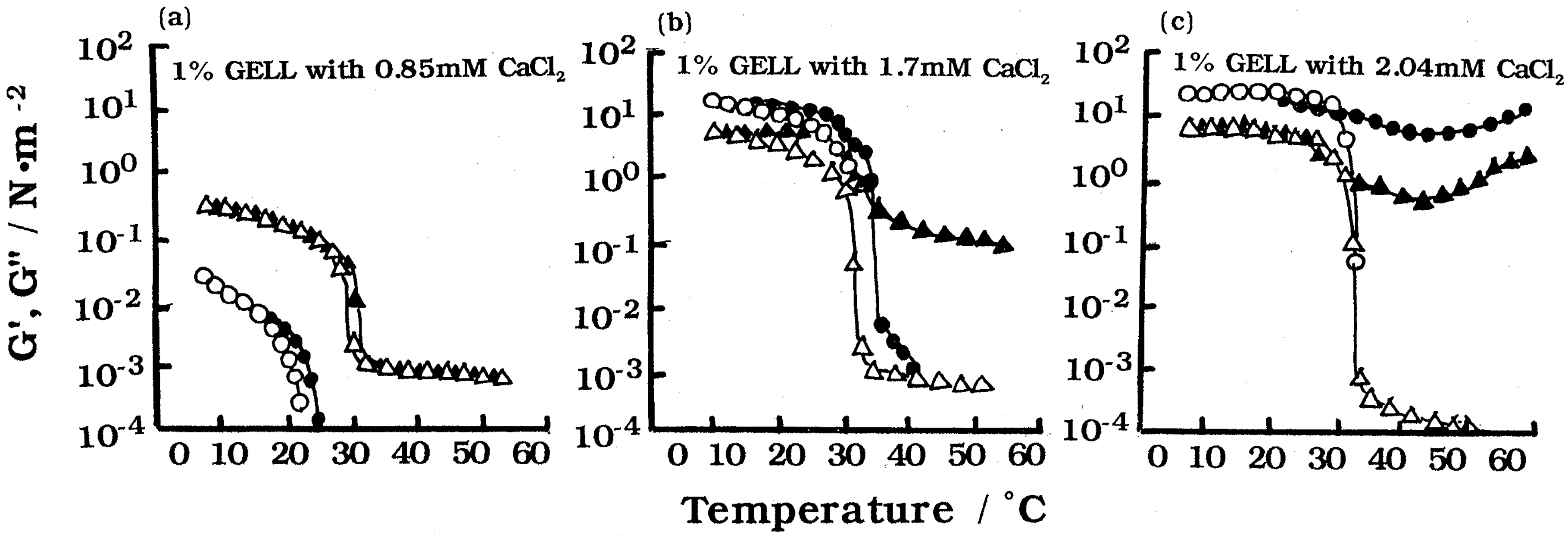

Fig. III-1 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 0.8% gellan gum solution containing CaCl$_2$ of various concentrations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate, 0.5℃/min; freq.ω=0.1rad/s. CaCl$_2$ conc.: (a), 0.85mM; (b), 1.7mM; (c), 2.04mM.

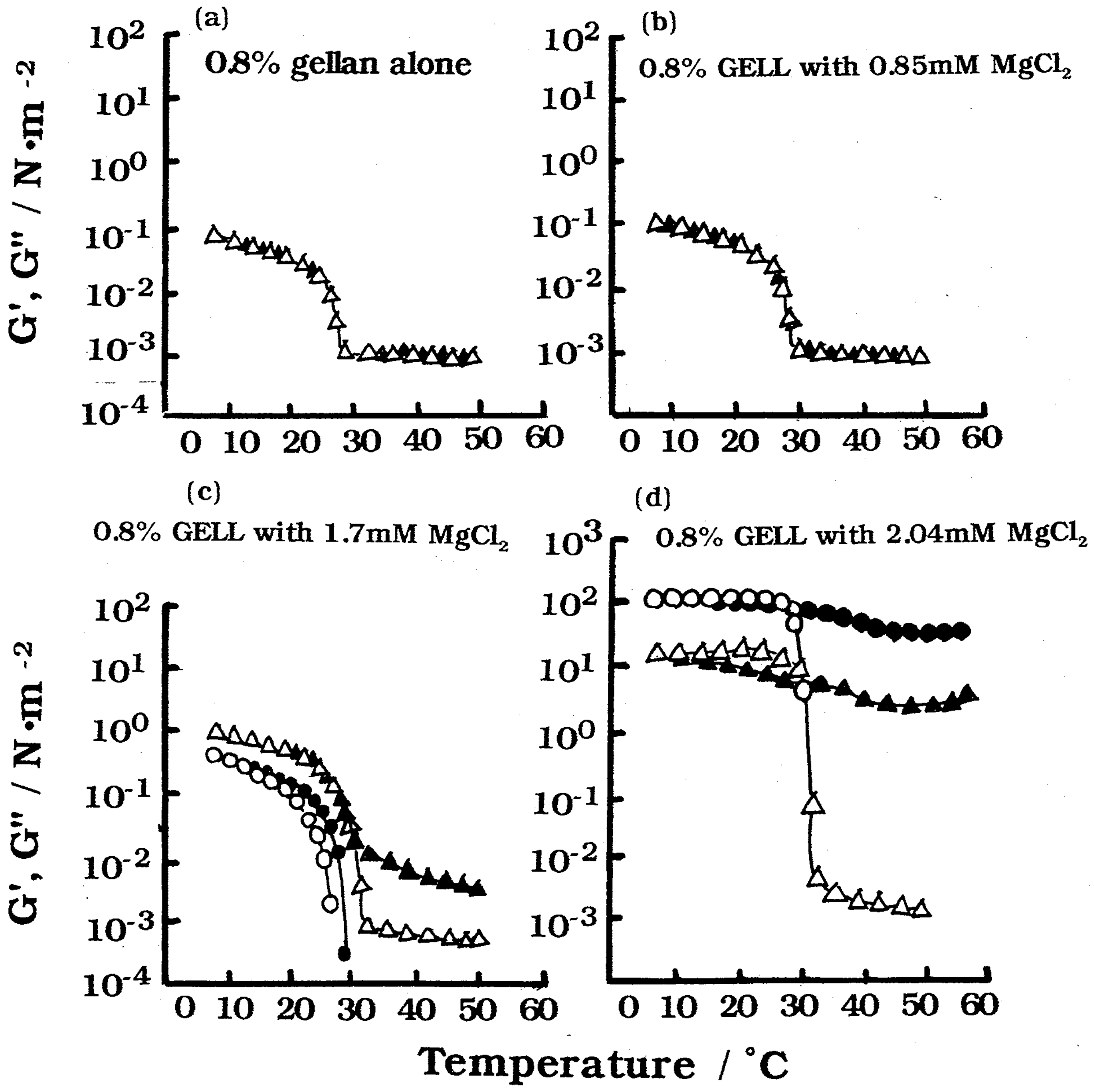

Fig. II-12 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 0.8% gellan gum solution containing MgCl$_2$ of various concentrations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s; MgCl$_2$ conc., 0mM(a), 0.85mM(b), 1.7mM(c), 2.04mM(D).

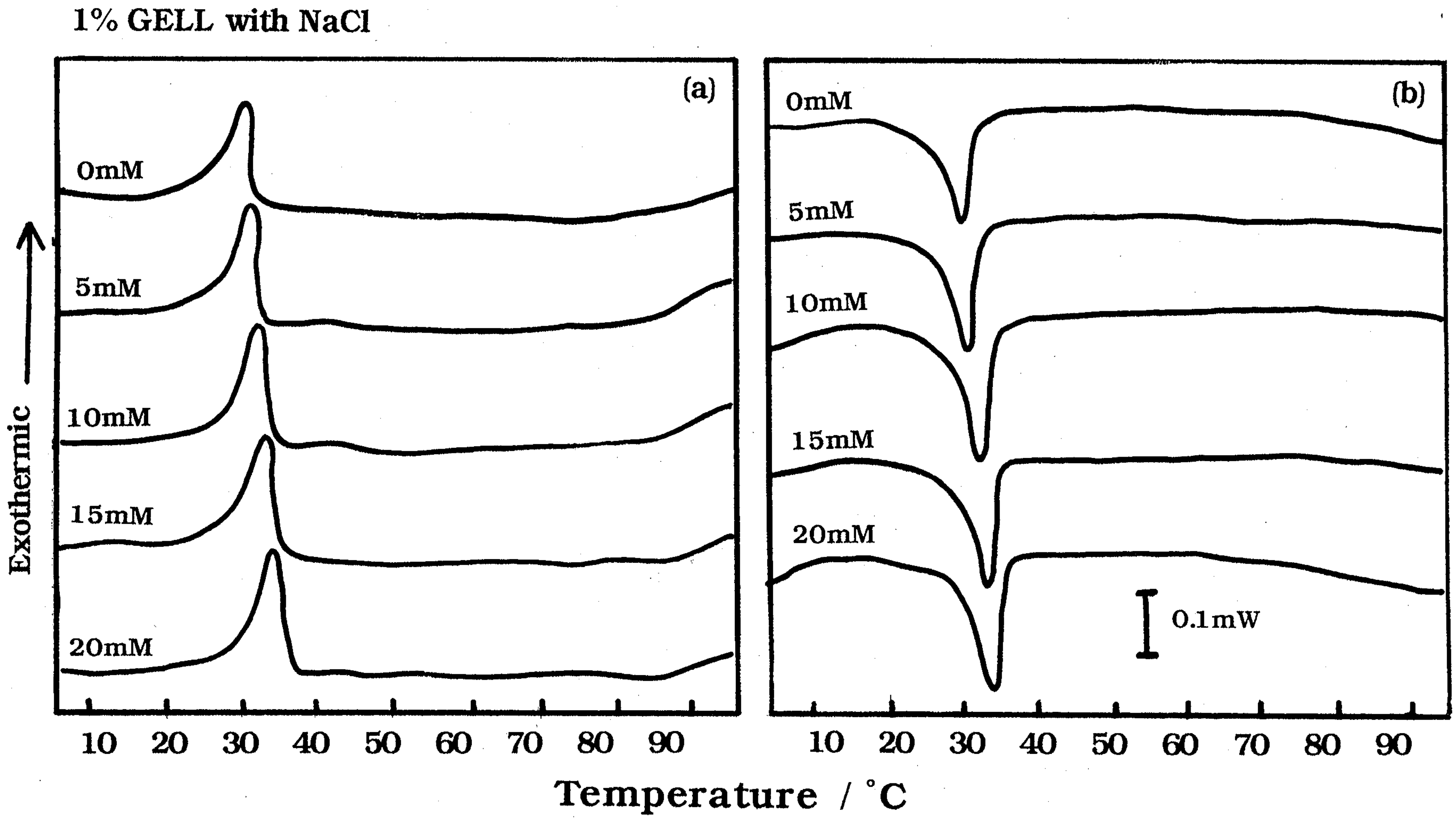

Fig. II-13 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing NaCl of various concentrations. Figures beside each curve represent the concentration of NaCl.
Cooling or heating rate ; 0.5℃/min.

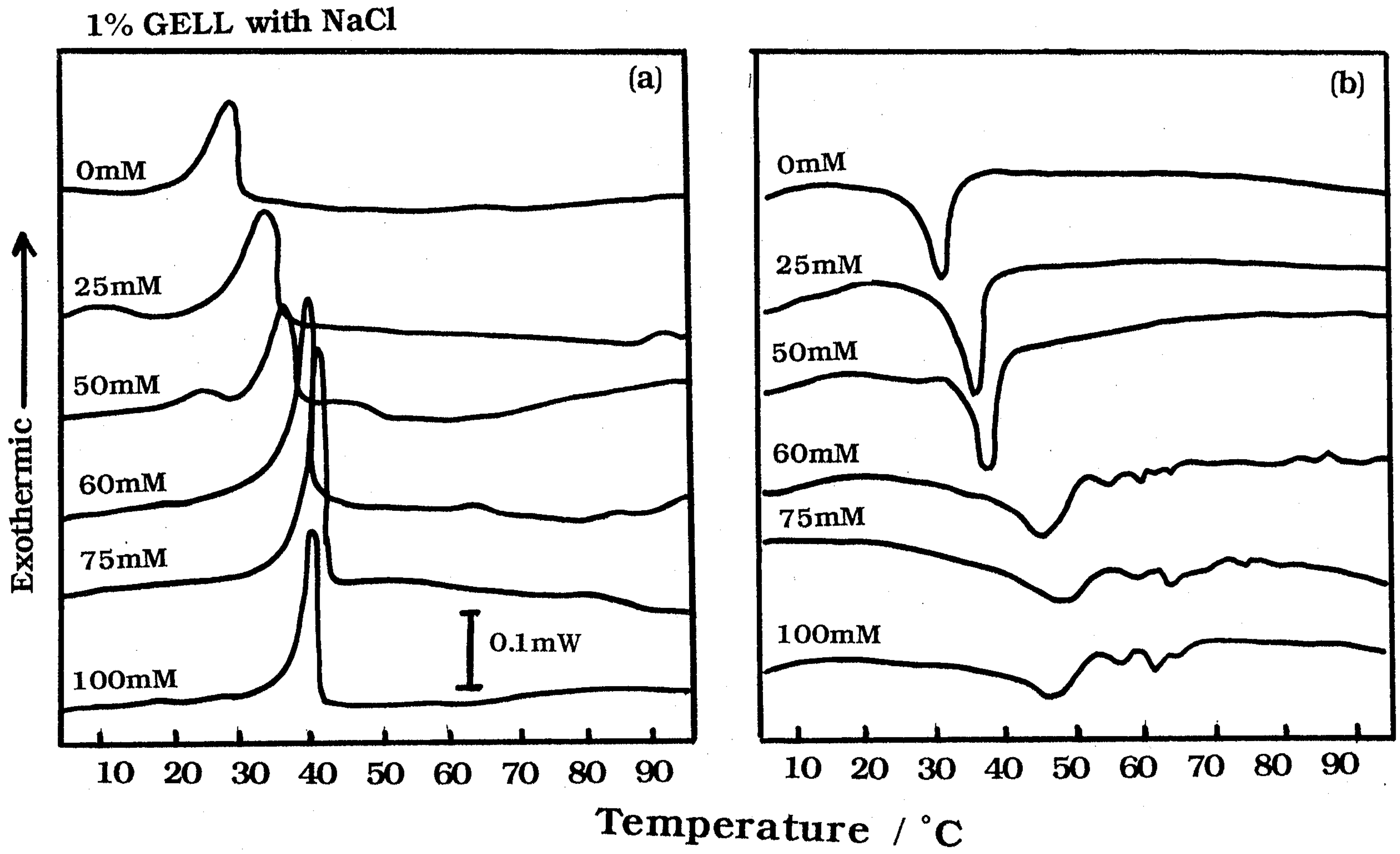

Fig. II-14 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing NaCl of various concentrations. Figures beside each curve represent the concentration of NaCl. Cooling or heating rate ; 0.5℃/min.

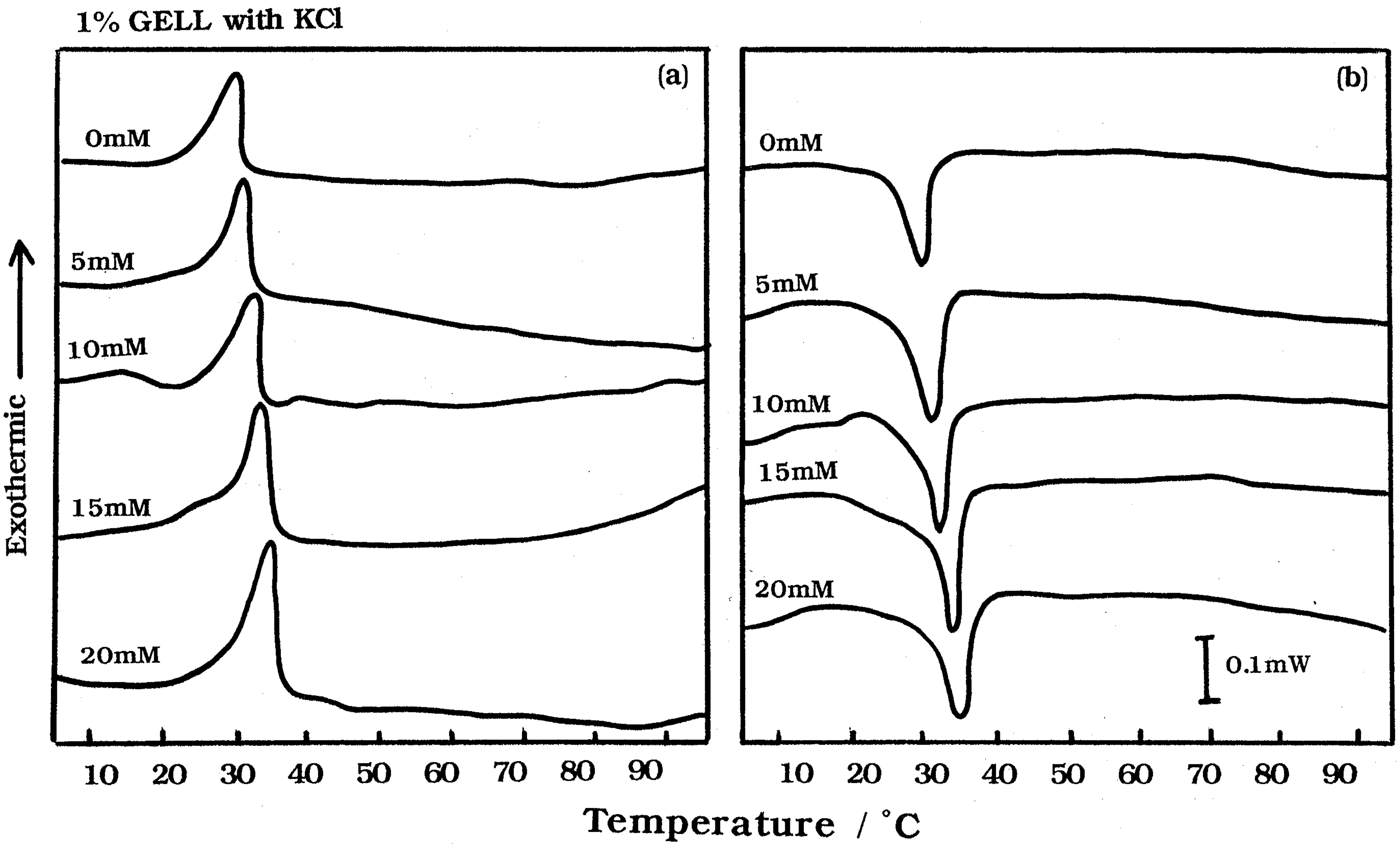

Fig. II-15 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing KCl of various concentrations. Figures beside each curve represent the concentration of KCl.
Cooling or heating rate ; 0.5℃/min.

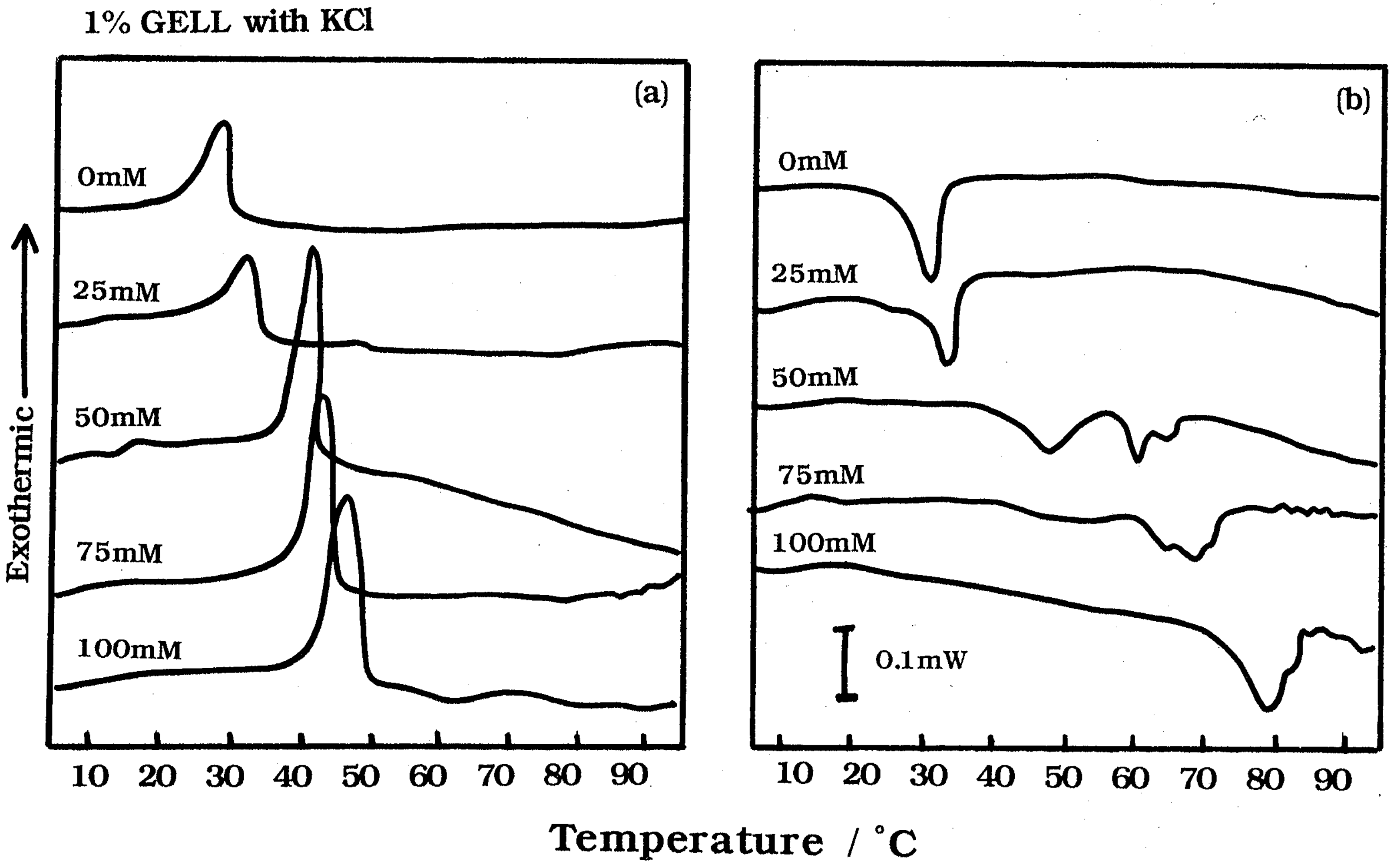

Fig. II-16 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing KCl of various concentrations. Figures beside each curve represent the concentration of KCl. Cooling or cooling rate ; 0.5℃/min.

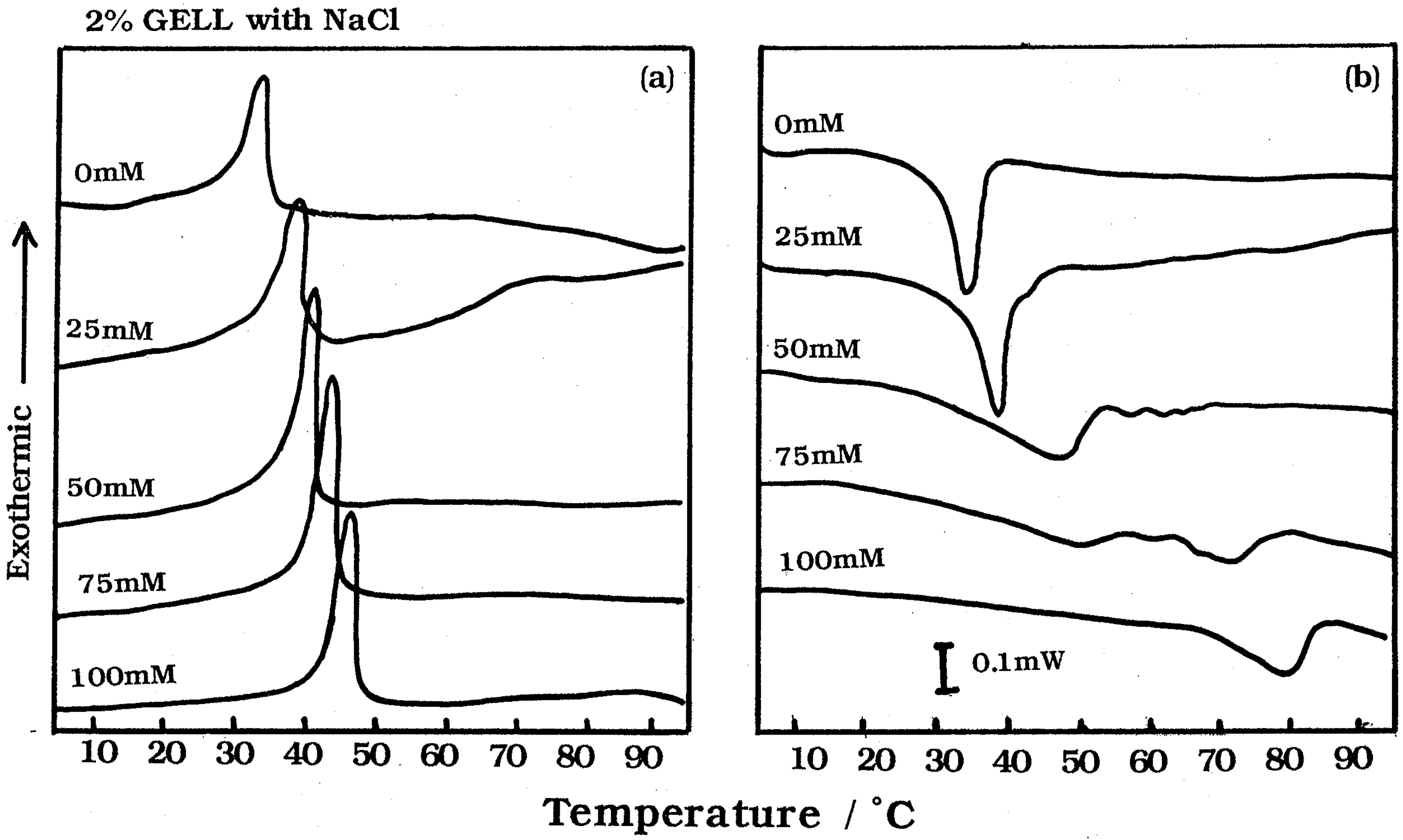

Fig. II-17 Cooling (a) and heating (b) DSC curves for 2% gellan gum solutions containing NaCl of various concentrations. Figures beside each curve represent the concentration of NaCl.
Cooling or heating rate ; 0.5℃/min.

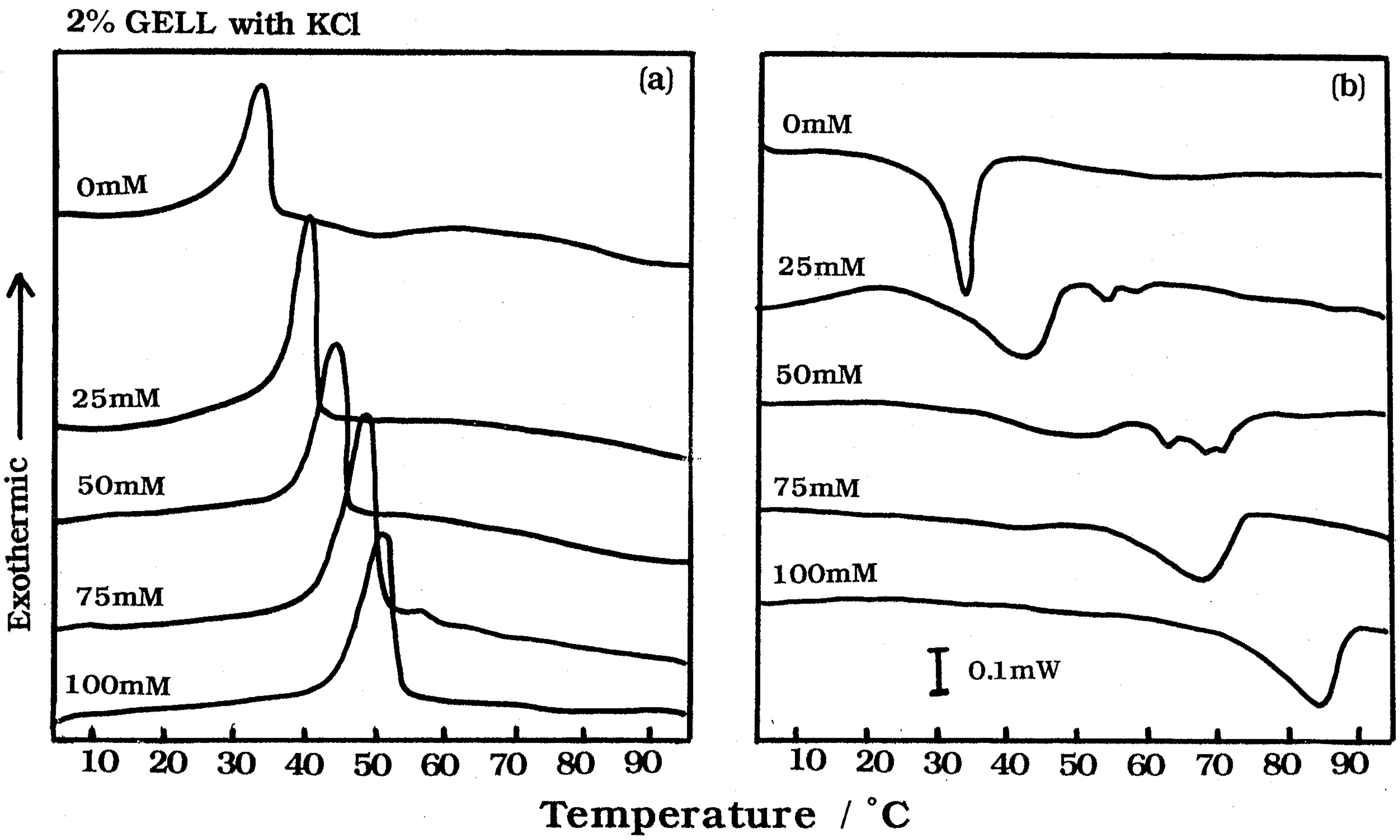

Fig. II-18 Cooling (a) and heating (b) DSC curves for 2% gellan gum solutions containing KCl of various concentrations. Figures beside each curve represent the concentration of KCl.
Cooling or heating rate ; 0.5℃/min.

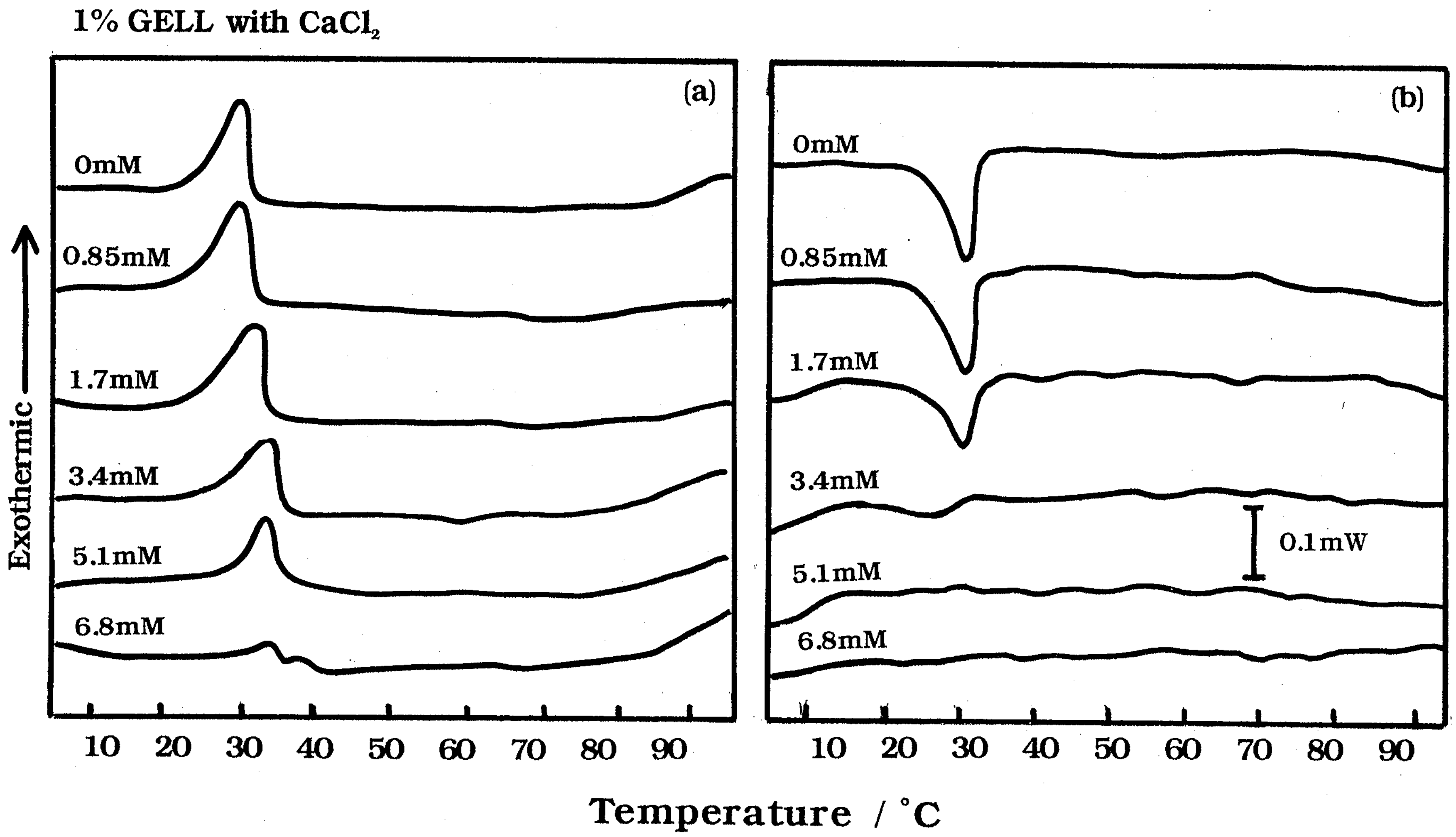

Fig. II-19 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing CaCl$_2$ of various concentrations. Figures beside each curve represent the concentration of CaCl$_2$. Cooling or heating rate ; 0.5℃/min.

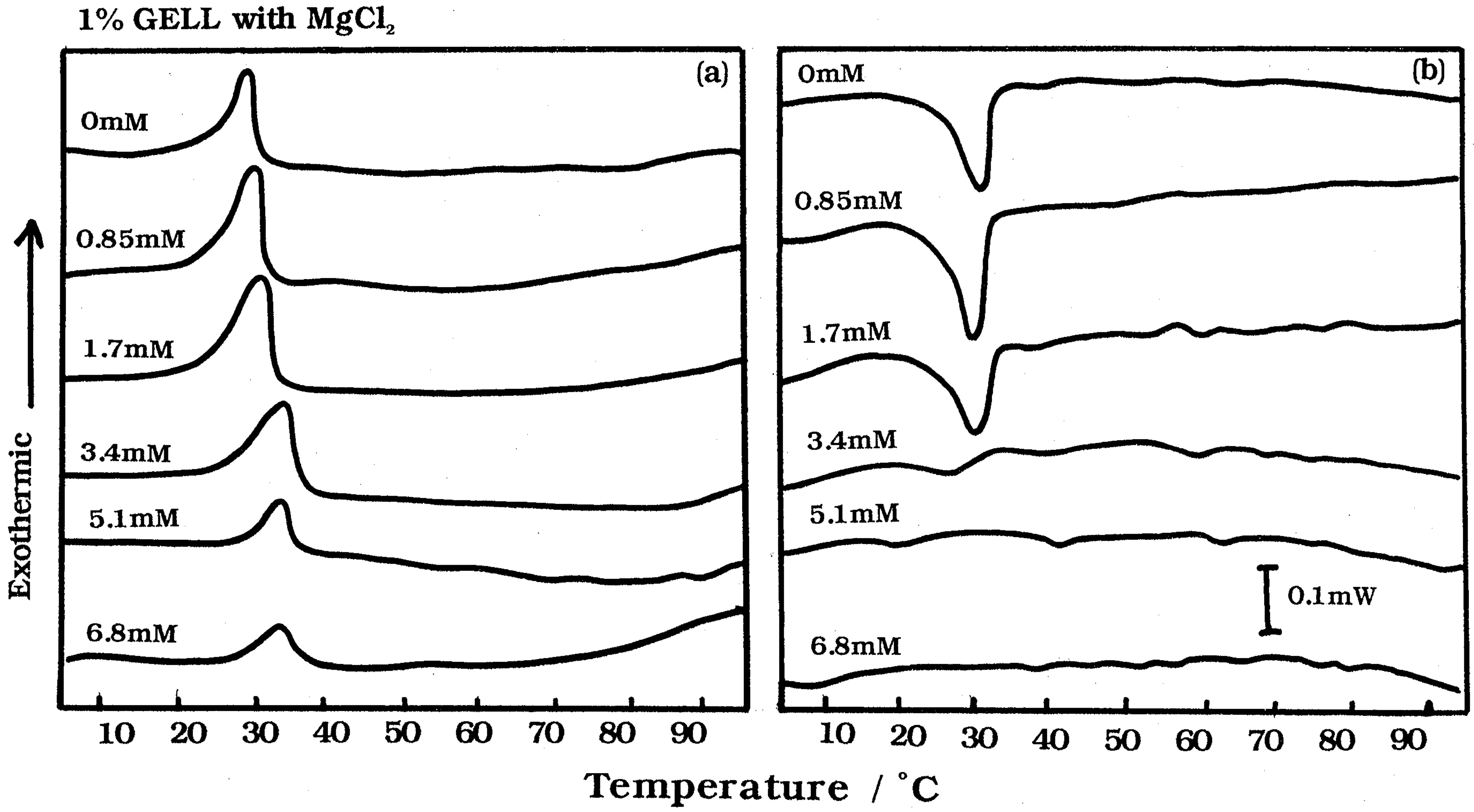

Fig. II-20 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing $MgCl_2$ of various concentrations. Figures beside each curve represent the concentration of $MgCl_2$. Cooling or heating rate ; 0.5℃/min.

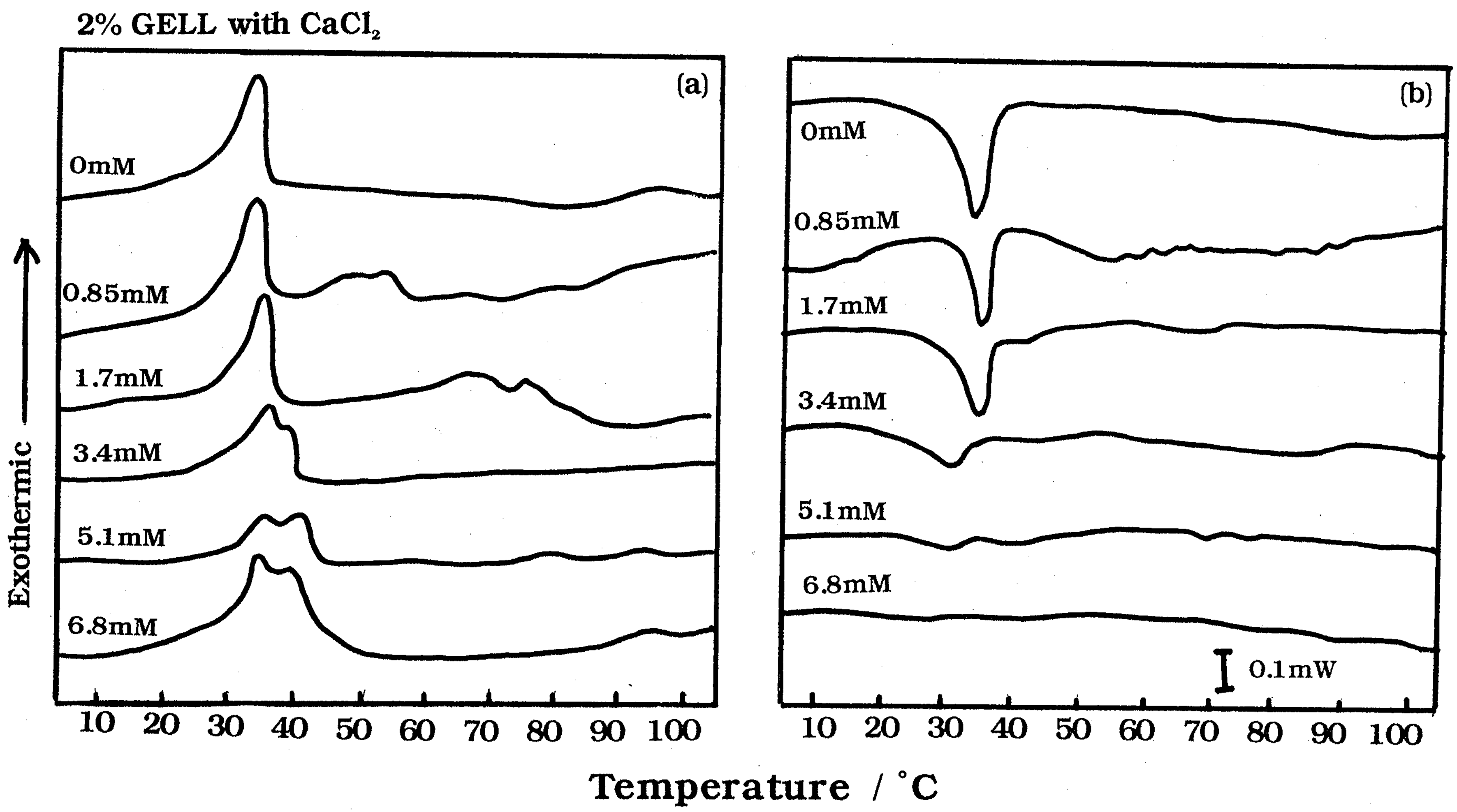

Fig. I-21 Cooling (a) and heating (b) DSC curves for 2% gellan gum solutions containing CaCl$_2$ of various concentrations. Figures beside each curve represent the concentration of CaCl$_2$. Cooling or heating rate ; 0.5℃/min.

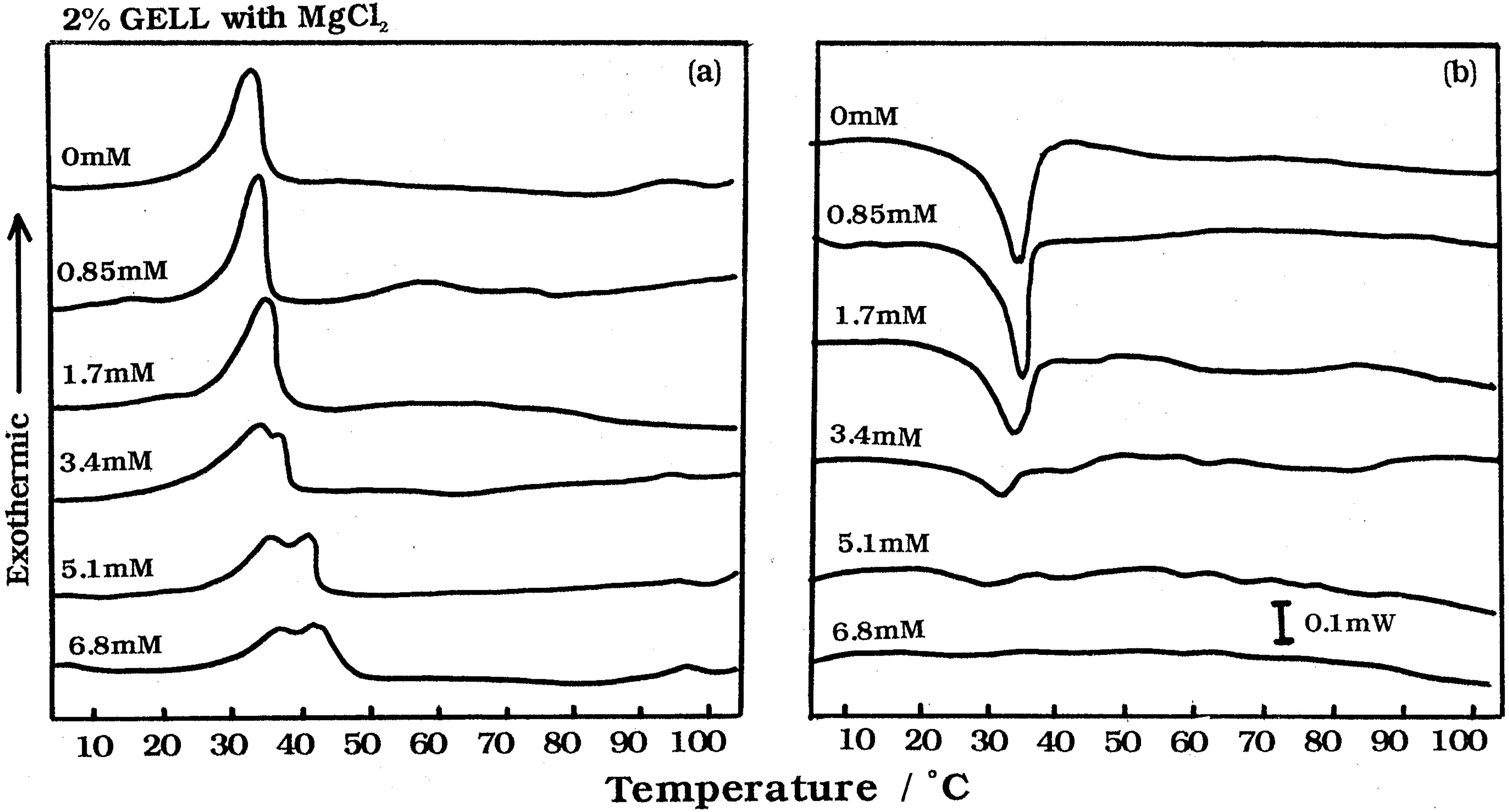

Fig. II-22 Cooling (a) and heating (b) DSC curves for 2% gellan gum solutions containing $MgCl_2$ of various concentrations. Figures beside each curve represent the concentration of $MgCl_2$. Cooling or heating rate ; 0.5℃/min.

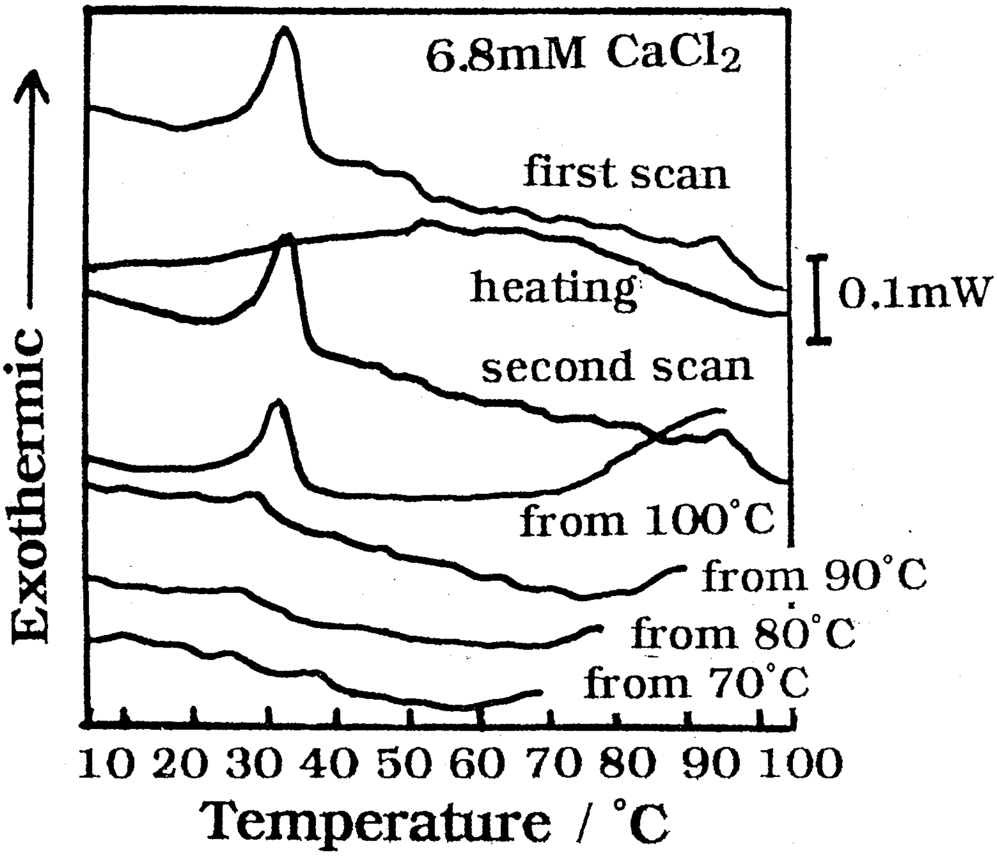

Fig. II-23 Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 6.8mM $CaCl_2$. Figures beside each curve represent the initial heating temperature. Cooling or heating rate ; 0.5°C /min.

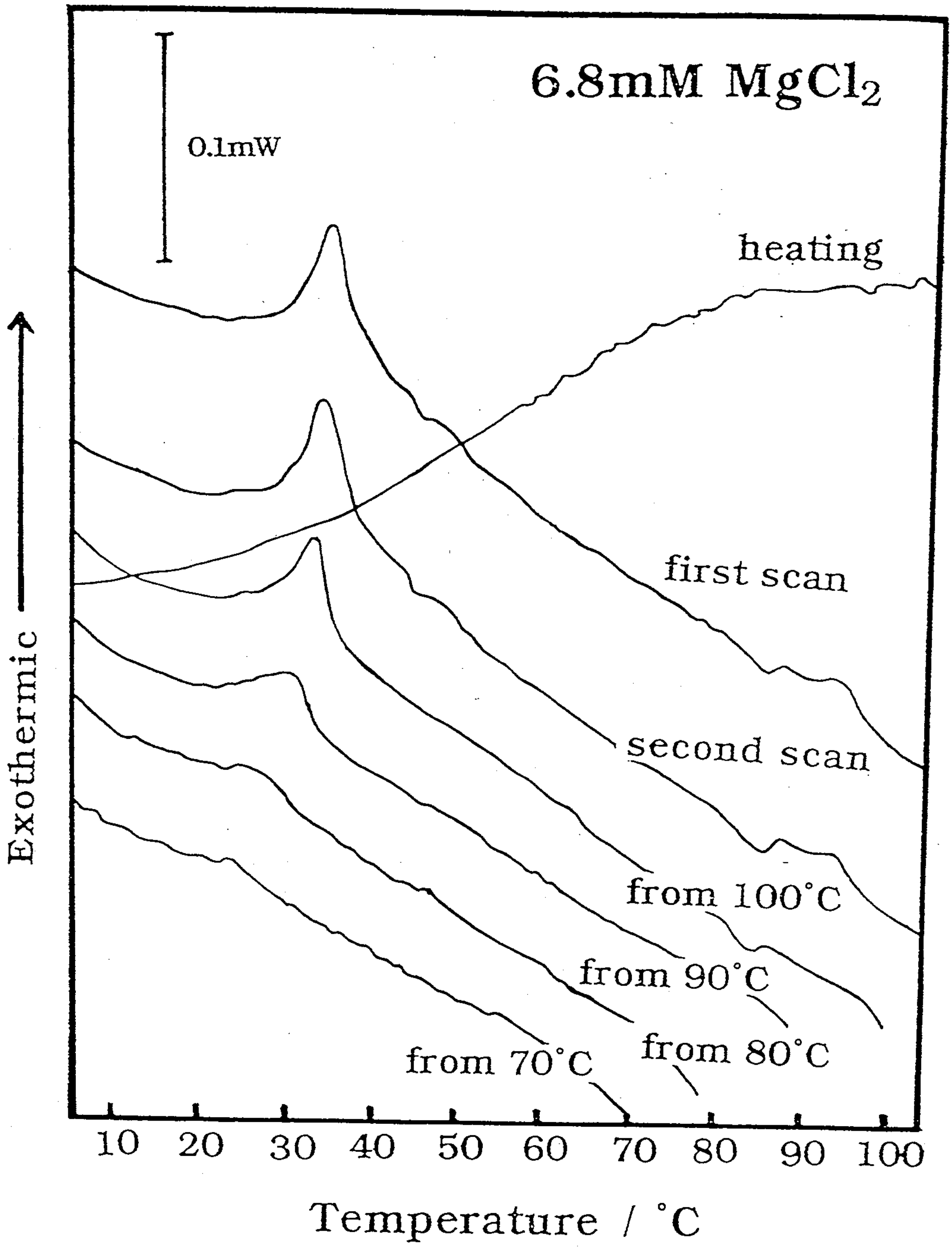

Fig. II-24 Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 6.8mM MgCl$_2$. Figures beside each curve represent the initial heating temperature. Cooling or heating rate; 0.5℃/min.

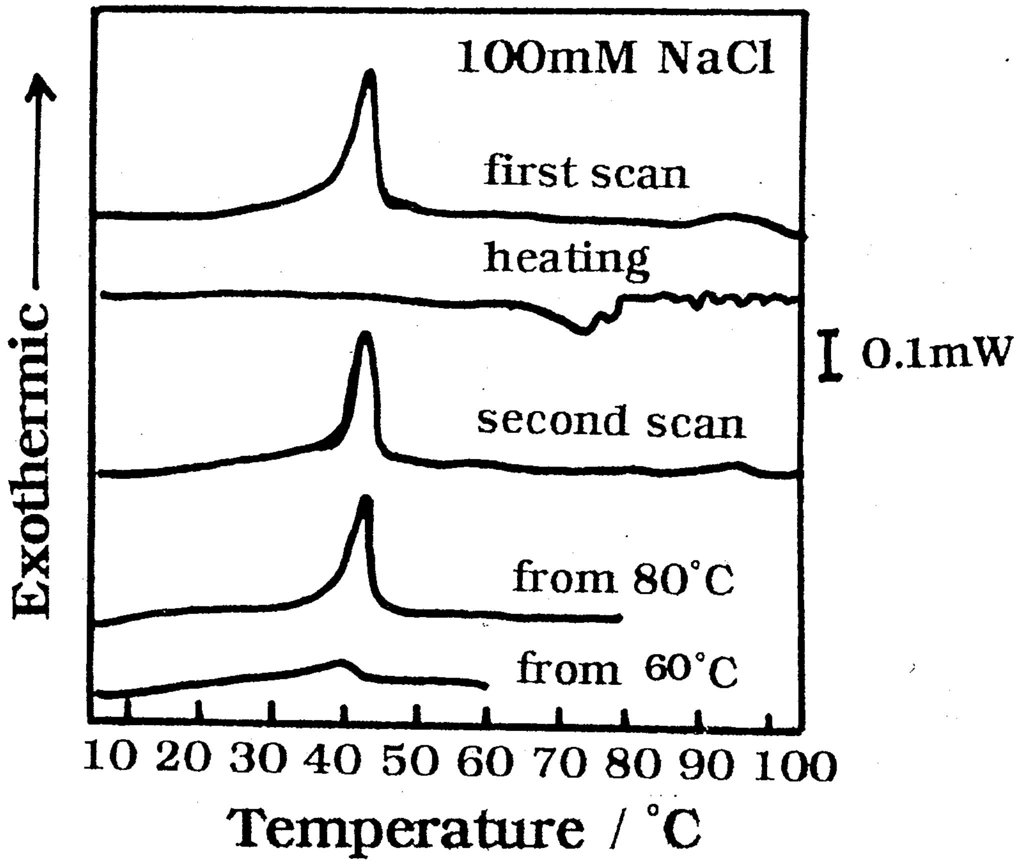

Fig. II-25 Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 100mM NaCl. Figures beside each curve represent the initial heating temperature. Cooling or heating rate ; 0.5℃/min.

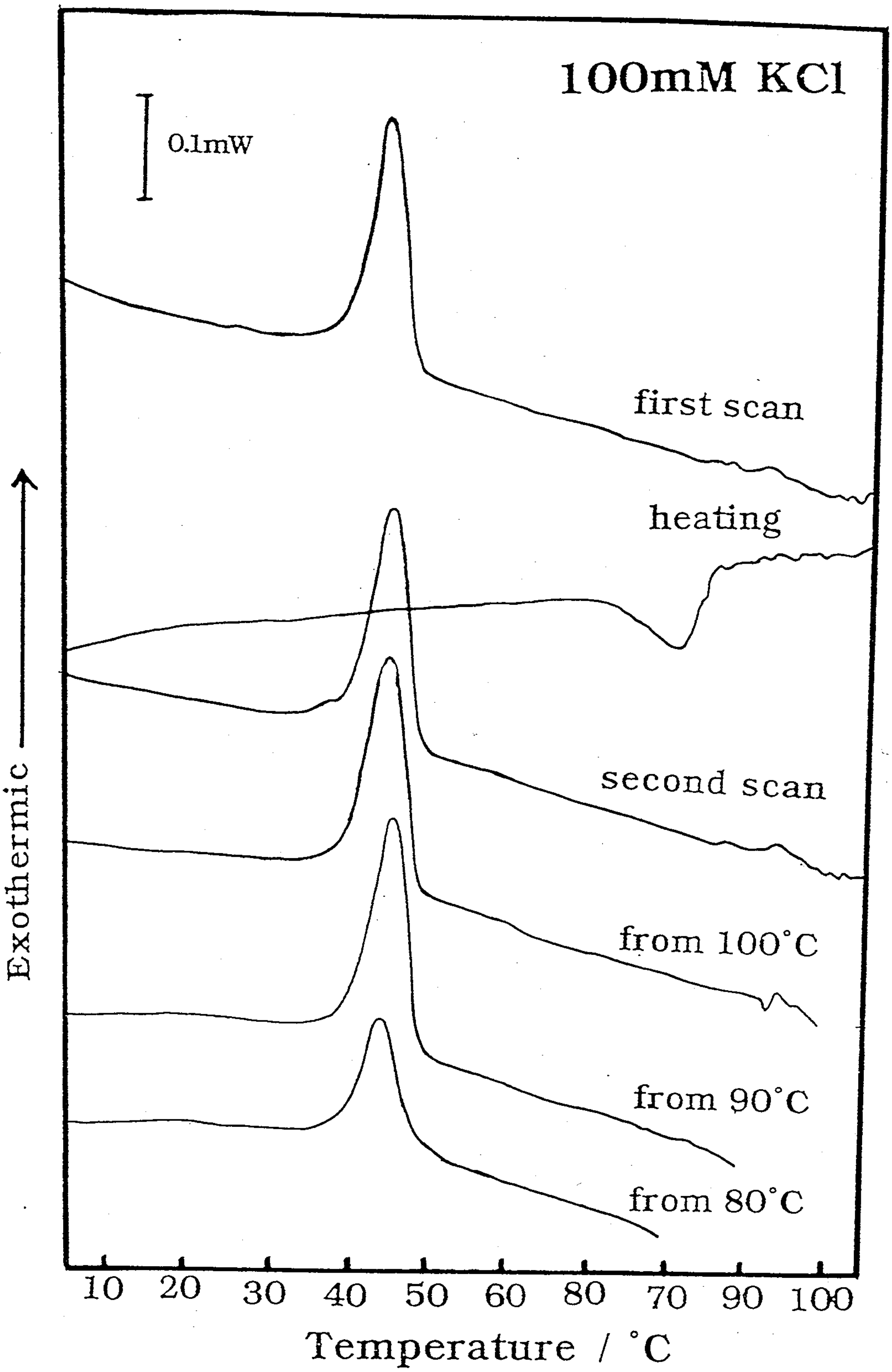

Fig. II-26 Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 100mM KCl. Figures beside each curve represent the initial heating temperature. Cooling or heating rate; 0.5℃/min.

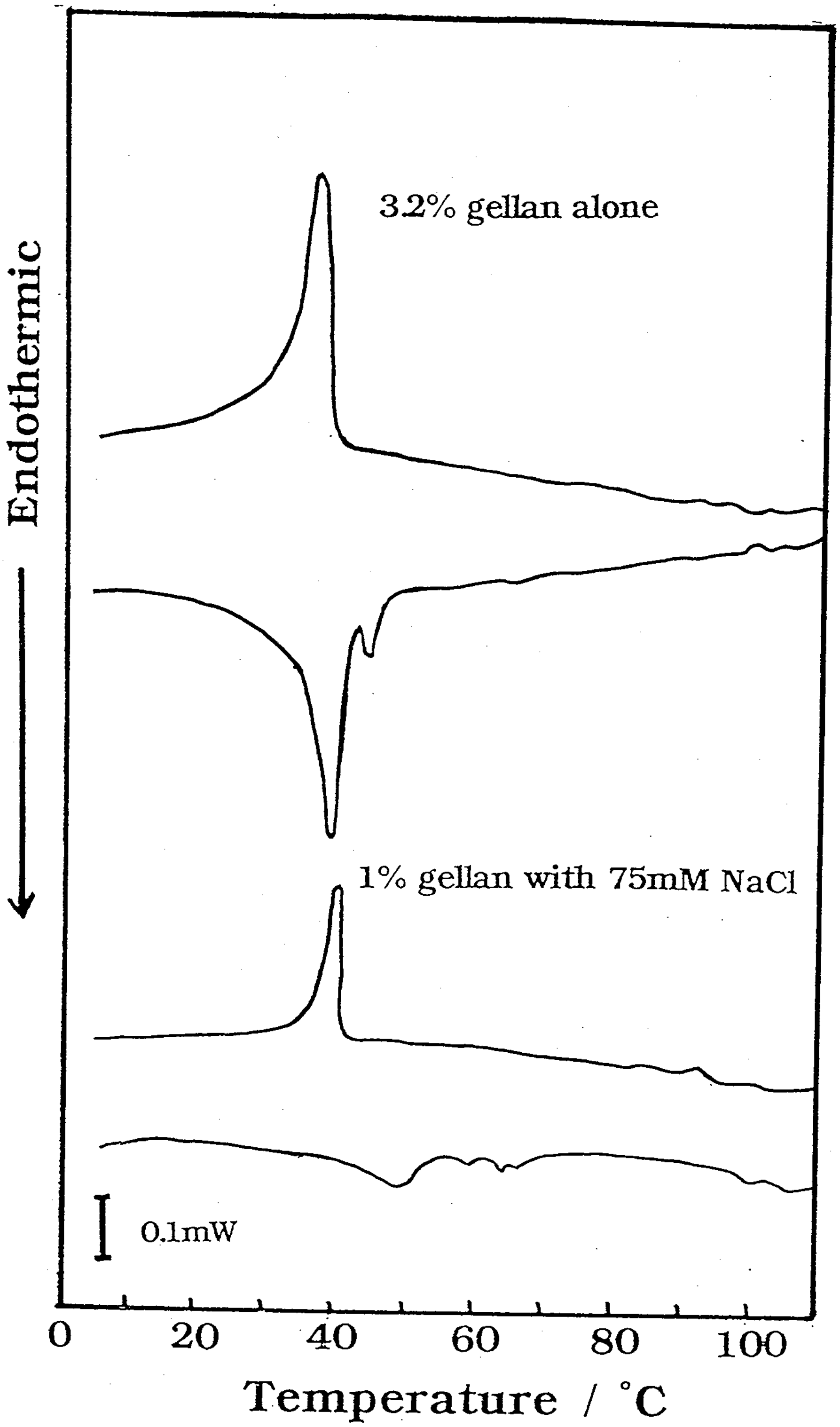

Fig. II-27 Cooling and heating DSC curves for a 3.2% gellan gum solution without salt and of a 1% gellan gum solution with 75mM NaCl. Cooling and heating rate:0.5℃/min.

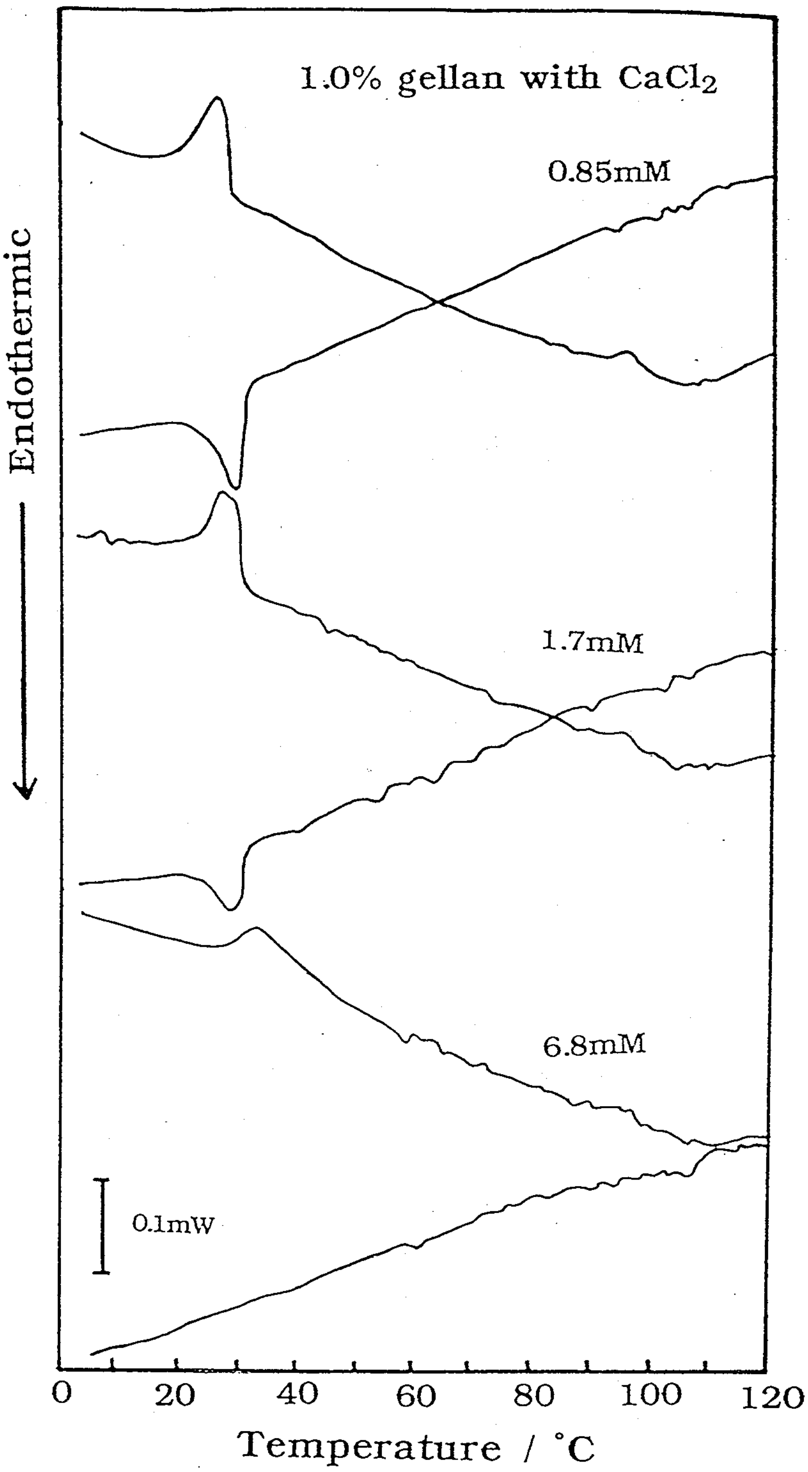

Fig. II-28 Cooling and heating DSC curves for 1% gellan gum solutions containing $CaCl_2$ of various concentrations. Figures beside each curve represent the concentration of $CaCl_2$. Cooling and heating rate:0.5℃/min.

III. Effects of Sugars and Konjac Glucomannan with Low Molecular Weights on the Sol-Gel Transition in Gellan Gum Aqueous Solutions.

MATERIALS AND METHODS

Gellan gum used in this chapter was the same as described in Chapter I. Glucose, sucrose, and mannose used in this study were of the extra fine grade reagents (Wako Pure Chemical Industries Ltd., Osaka, Japan), and were used without further purification. Three fractions of konjac glucomannan with different molecular weights (LM-1, LM-3 and LM-4), prepared by enzymatic degradation, were kindly supplied by Shimizu Chemical Co., Hiroshima, Japan. Molecular weights of these fractions determined by the same method by Gel Permeation Chromatography were 9.5×10^5 (LM-1), 1×10^5 (LM-3) and 1×10^4 (LM-4).

The gellan gum solutions were prepared in the same way as described in section 2 of Chapter I. In the case of samples containing sugars, the concentration of glucose, sucrose or mannose in solutions varied from 0~4.0M, and each sugar was added to 1.0% or 0.5% gellan gum solution during stirring to swell before heating. In the case of samples added to konjac glucomannan, the concentration of gellan gum was fixed constant at 1.0 or 0.5% and konjac glucomannan was added at a concentration progressively increasing from 0% to 4.0%. Methods of rheological and DSC measurements were described in section 2 of Chapter I.

RESULTS

Rheological properties

Figure III-1 (a)~(f) shows the temperature dependence of G' and G'' during cooling and heating process for a 0.5% gellan gum solution containing glucose of various concentrations at a constant frequency of 0.1rad/s and at a cooling or heating rate of 0.5℃/min. For a 0.5% gellan gum solution without sugar [Figure III-1(a)], G' was too small to be detected in both cooling and heating processes because the value was much more erratic, however, G'' showed a one step-like change at 22℃, which was attributed to the helix-coil transition temperature T_{hc} in gellan gum molecules, as described above. In the presence of 0.05M glucose [Figure III-1(b)], the values of G'' at higher temperature sides slightly decreased rather than those without sugar, however, T_{hc} shifted to a higher temperature and then the values of G" at lower temperature sides significantly increased than those without sugar. Although T_{hc} shifted to higher temperatures with increasing concentration of glucose, G' began to be detected during cooling and heating processes in addition of >1.2M glucose [Figure III-1(c)], and both moduli gradually increased with increasing concentration of glucose (Figure III-1(d)). Upon addition of 3M glucose [Figure III-1(e)], the cross-over of G' and G" was observed at 7℃, which was attributed to the sol-gel transition temperature T_{sg}. Both T_{hc} and T_{sg} significantly shifted to higher temperatures with increasing concentration of glucose [Figure III-1(f)].

Figure III-2 (a)~(f) shows the temperature dependence of G' and G" during cooling and heating process for a 1.0% gellan gum solution containing glucose of various concentrations at 0.1rad/s at a cooling or heating rate of 0.5℃/min. For a 1.0% gellan gum solution without sugar [Figure III-2(a)], although G' was too small to be detected in both cooling and heating processes, G" showed a one step-like change at 30℃ corresponding to T_{hc}, described above. Upon addition of 0.01M [Figure III-2(b)], G' could be detected in the cooling and heating processes, and T_{hc} shifted to higher temperatures, however, G" at higher temperature sides slightly decreased rather than that without sugar. With progressive addition of glucose, G' gradually approached G", and then upon addition of 1.6M glucose [Figure III-2(c)], the cross-over of G' and G" was observed around 7℃ corresponding to T_{sg}. Both T_{hc} and T_{sg} shifted to higher temperatures with increasing concentration of added glucose. Upon addition of 1.8M glucose [Figure III-2(d)], the temperature dependence of G' showed a larger hysteresis, and the thermal hysteresis was gradually modified with increasing concentration of added glucose [Figure III-2(e)]. Upon addition of 3.5M glucose [Figure III-2(f)], T_{hc} and T_{sg} was observed concurrently in cooling process, however, in the heating process, both G' and G" showed only a small decrease, so that the marked difference between G" in cooling and heating processes was observed at higher temperature sides.

Figure III-3 (a)~(d) shows the temperature dependence of G' and G" during cooling and heating process for a 0.5% gellan gum solution containing sucrose of various concentrations at 0.1rad/s at a cooling or heating rate of 0.5℃/min. Upon addition of > 0.8M sucrose, G' could be detected in the cooling and heating process [Figure III-3(a)], the minimum molar concentration of sugars above which G' could be detected was far lower for sucrose than for glucose (1.2M) [Figure III-1(a)]. Both moduli gradually increased and T_{hc} shifted to higher temperatures with increasing concentration of sucrose [Figure III-3(b)]. Upon addition of >1.5M sucrose [Figure III-3(c)], the cross-over of both moduli was observed, and T_{sg} significantly shifted to higher temperatures with increasing concentration of sucrose [Figure III-3(d)].

Figure III-4 (a)~(f) shows the temperature dependence of G' and G" during cooling and heating process for a 1.0% gellan gum solution containing sucrose of various concentrations at a constant frequency of 0.1rad/s and at a cooling or heating rate of 0.5℃/min. As shown in Figure III-2(a), for a 1.0% gellan gum solution without sugar, G' was too small to be detected in both cooling and heating processes, however, G' could be detected by addition of 0.01M sucrose [Figure III-4(a)]. Although both G' and G" increased with increasing concentration of sucrose (Figure III-4(b)), G' gradually approached G", eventually, the cross-over of both moduli was observed at 6.7 ℃ for a 1% solution containing 1.1M sucrose [Figure III-4(c)]. With more progressive addition of sucrose, T_{hc} gradually increased, however, T_{sg} significantly shifted to higher temperatures with increasing concentration of sucrose [Figure III-4(d)]. The temperature dependence of both moduli upon addition of 1.6M sucrose showed a larger hysteresis; both G' and G" in cooling process showed a drastic increase at a certain temperature, in contrast, both moduli did not decrease so much in the heating process [Figure III-4(e)]. Upon addition of 1.8M sucrose [Figure III-4(f)], although both moduli showed a drastic increase at 50℃ in cooling process, there was no evidence for drastic decrease of both moduli up to 70℃ in the heating process.

Figure III-5 (a)~(d) shows the temperature dependence of G' and G" during cooling and heating process for a 0.5% gellan gum solution containing mannose of various concentrations at 0.1rad/s at a cooling or heating rate of

0.5℃/min. Upon addition of 0.5M mannose [Figure III-5(a)], G' could be detected in cooling and heating process, and then G' gradually approached G" with increasing concentration of mannose [Figure III-5(b) and(c)]. However, even in addition of 3M mannose [Figure III-5(d)], G" remained larger than G' throughout temperature range from 60℃ to 5℃, and the cross-over of both moduli could not be observed. Figure III-1-6 (a)~(f) shows the temperature dependence of G' and G" during cooling and heating process for a 1.0% gellan gum solution containing mannose of various concentrations at 0.1rad/s at a cooling or heating rate of 0.5℃/min. Upon addition of only 0.01M mannose [Figure III-6(a)], G' could be detected in cooling and heating processes, as in the presence of glucose or sucrose. Both G' and G" increased and T_{hc} shifted to higher temperatures with increasing concentration of mannose [Figure III-6(b) and (c)]. Upon addition of 2.0M mannose [Figure III-6(d)], the cross-over of both moduli was observed at 5℃ corresponding to T_{sg}. T_{sg} shifted to higher temperatures with increasing concentration of mannose [Figure III-6(e) and (f)].

As described above, the helix-coil transition temperature T_{hc} is defined as the temperature at which G" changes most steeply, and the sol-gel transition temperature T_{sg} is defined as the temperature at which G' and G" showed a cross-over. The main contribution of the magnitude of the relaxational strength ΔG should be by the formation and the subsequent aggregation of helices of gellan molecules, as discussed above.

Figure III-7 shows T_{hc} and T_{sg} and ΔG for 1% gellan gum solutions as a function of concentration of added glucose, sucrose or mannose. In the presence of glucose [Figure III-7(a)], T_{hc} and T_{sg} monotonically shifted to higher temperatures with increasing concentration of added glucose, eventually, in the presence of 3.5M glucose, T_{sg} almost coincided with T_{hc}. ΔG in the presence of glucose of fairly low concentration was slightly larger than that without sugar, and then showed a maximum around 1.8M glucose [Figure III-7(b)]. Sucrose shifted most effectively T_{hc} and T_{sg} to higher temperatures with comparison to glucose and mannose. In the presence of sufficient sucrose, although T_{sg} probably coincided with T_{hc}, the concentration at which T_{sg} coincided with T_{hc} is lower than that in the presence of glucose. ΔG as a function of sucrose [Figure III-7(d)] showed a maximum at 1.6M sucrose, this concentration was slightly lower than that in the presence of glucose [Figure III-7(b)]. In the case of added mannose [Figure III-7(e)], although T_{hc} and T_{sg} shifted to higher temperatures with increasing concentration of added mannose, Thc remained significantly higher than Tsg up to 3M mannose added. ΔG in the presence of mannose [Figure III-7(f)] showed a plateau value to increasing concentration of mannose, although ΔG in the presence of mannose of fairly low concentration was slightly smaller compared to that without sugar.

As illustrated in Figure III-1~III-7, G' and G'' increased by addition of all these sugars in the order of effectiveness: sucrose>glucose>mannose. This order is in good agreement with the dynamic hydration number [n_{DHN}(glucose)=18.6; n_{DHN}(sucrose)=25.2; n_{DHN}(mannose)=14.7] and the number of equatorially attached OH groups [n(e-OH)(glucose)=4.6; n(e-OH)(sucrose)=6.3; n(e-OH)(mannose)=3.3]. It has been well known that there was a good correlation between the dynamic hydration number and the n(e-OH) of saccharides (Uedaira and Ishimura, 1989; Uedaira and Ohsaka, 1991). Similar relations have been observed for agarose (Watase et al., 1992; Nishinari et al., 1995) and κ-carrageenan (Nishinari and Watase, 1990; Nishinari et al., 1995) gels.

For 0.5% or 1.0% gellan gum solutions with added sugars, the differences $\Delta T_{hc}=T_{hc}-T_0$ per unit concentration of added sugars,(where T_0 is the helix-coil transition temperature T_{hc} of gellan gum alone), as a function of n(e-OH) are shown in Figure III-8(a) and (b). These relationships seem to be linear within the experimental errors, which indicates that the stability of gellan gum gels relates with n(e-OH). The least-squares fitting leads to the following relations:

$\Delta T_{hc}/C_s=1.73n(e\text{-}OH)-3.6$ (K · l/mol)

for 0.5% gellan gum (Figure III-8(a))

$\Delta T_{hc}/C_s=1.74n(e\text{-}OH)-3.7$ (K · l/mol)

for 1.0% gellan gum [Figure III-8(b)], where C_s is the concentration of added sugar.

Figure III-9 (a)~(f) shows T_{hc} and ΔG for 1% gellan gum solutions as a function of concentration of added LM-1, LM-3 or LM-4, compared to those in the presence of sugars. As shown above [Figure III-7(a), (c) and (e)], sugars significantly increased T_{hc}, however, T_{hc} as a function of KGM content didnot change so much [Figure III-9(a), (c), (e)]. ΔG as a function of KGM content was dependent on molecular weight of KGM. Hence, ΔG as a function of LM-1 with relatively higher molecular weight showed a monotonical decrease[Figure III-9(b)], however, ΔG upon addition of LM-3 [Figure III-9(d)] or LM-4 [Figure III-9(f)] with relative lower molecular weight significantly increased and then didnot change so much with increasing content of KGM.

Figure III-10 (a)~(c) shows G' of 1% gellan gum solution as a function of concentration of added sugar: glucose, sucrose or mannose at 0.1rad/s at various temperatures from 30℃ to 10℃. As shown in Figure III-10(a), at any temperature, G' as a function of glucose concentration showed a monotonical increase. As shown in Figure III-10(b), G' markedly increased up to a certain concentration of sucrose and then showed plateau or slightly decreased. As shown in Figure III-10(c), G' as a function of mannose concentration showed a complicated behaviour; G' at any temperature increased with increasing concentration of mannose up to 0.6M or 0.7M but decreased on addition of 1.0M or 1.2M mannose, and then increased again with increasing concentration of added mannose.

DSC

Figure III-11 shows cooling and heating DSC curves for 1% gellan gum solutions containing glucose of various concentrations. The cooling or heating DSC curves for a 1% solution of gellan gum alone showed a single exothermic or endothermic peak around 30℃, and these peaks were attributed to the helix-coil transition in GELL molecules. Upon addition of glucose of fairly low concentration, the exothermic peak temperature T_s and endothermic peak temperature T_m slightly shifted to lower temperatures and exothermic and endothermic enthalpies significantly decreased compared to those without sugar. However, with further addition of glucose, T_s and T_m gradually shifted to higher temperatures. The exothermic and endothermic enthalpies slightly increased up to a certain concentration of glucose, and then monotonically decreased with increasing concentration of glucose. In the presence of 1.8M glucose, although the main exothermic peak in the cooling DSC curve was relatively sharper, the heating DSC curve did not show a clear peak. In the presence of sufficient glucose (3M), not only the heating DSC curve but also the cooling DSC curve became much broader, however, the cooling DSC curve showed many small exothermic peaks at both higher and lower temperatures. The change of DSC curves as a function of concentration of sucrose or mannose was similar to that as a function of glucose (Figure III-11), although the order of effectiveness was sucrose>glucose>mannose.

Figure III-12 shows T_s and T_m for 1% gellan gum solutions as a function of concentration of glucose, sucrose or mannose. In all the cases, both T_s and T_m in the presence of sugar of fairly low concentration slightly decreased compared to those without sugar, especially in the presence of mannose, these temperatures significantly shifted to lower temperatures. However, with more progressive addition of any sugar, both T_s and T_m monotonically shifted to higher temperatures. In the presence of sucrose [Figure III-12(b)], T_s and T_m containing >1M drastically shifted to higher temperatures, however, in the presence of sufficient sucrose both the exothermic and endothermic peaks became much broader, so that these temperatures could not be determined.

Figure III-13 shows the endothermic enthalpies ΔH_m for 1% gellan gum solutions as a function of concentration of glucose, sucrose or mannose. In the presence of glucose of fairly low concentration, ΔH_m was slightly smaller rather than those without sugar and then slightly increased with increasing concentration of glucose, however, it did not change so much compared to that without sugar [Figure III-13(a)]. In the presence of sucrose, although ΔH_m dropped by the addition of 0.05M sucrose, it gradually increased up to 1M sucrose, and then drastically increased with increasing concentration of added sucrose [Figure III-13(b)]. The curve of ΔH as a function of concentration of sucrose was in better agreement with that of G' rather than that of relaxational strength ΔG in the rheological measurement [Figure III-1-7(d)]. In the presence of added mannose, ΔH slightly decreased in the presence of mannose of fairly low concentration and it slightly increased up to a certain concentration of mannose, however, it decreased with more addition of mannose [Figure III-13(c)]. This behaviour was similar to ΔG as a function of mannose (Figure III-7).

Figure III-14 shows the exothermic and endothermic peak temperatures, T_s and T_m, and the endothermic enthalpies ΔH_m for 1% gellan gum solutions as a function of LM-3. Both T_s and T_m slightly decreased in the presence of LM-3 of low concentration and then levelled off with increasing concentration of LM-3. ΔHm didnot change so much with increasing concentration of LM-3. T_s, T_m or ΔH_m as a function of LM-1 or LM-2 concentration was similar to that as a function of LM-3. These results indicated that KGM little influenced the thermal stability of gellan gum solutions in comparison with sugars. However, KGM with relatively lower molecular weight significantly increased ΔG observed by the rheological measurement.

DISCUSSION

Since the junction zones of thermoreversible gels would be stabilized by weak molecular forces such as the electrostatic interactions, hydrogen bonds, hydrophobic interactions, the study on the effect of sugars, as well as salts, could provide important informations for the role of molecular forces in the stabilization of gel structure.

Effects of a sugar on the helix-coil transition of a polymer may be attributed to one of or both of the following factors; 1) the direct hydrogen bonding between hydroxyl groups in the polymer and in the sugar and/or 2) The structural change of water as solvent. Hence, sugar increases the number of elastically active network chains by forming hydrogen bonds or increases the effective concentration of GELL by immobilising water molecules. As shown in Figure III-10(a)~(c), in the presence of sufficient sugar, G' at any temperature became significantly larger than that without sugar, however, for a 3M solution of sugar alone (in the absence of GELL), G' could not be detected during both cooling and heating process (data not shown). This indicates that the large values of G' are induced by the interaction between GELL molecules and sugar molecules. However, since T_s, T_m and ΔH_m observed by DSC as a function of sugar concentration showed a slight decrease and the subsequent increase, it seems that the possibility of direct hydrogen bonding between hydroxyl groups in polymers and in sugars is more plausible at least when sugar concentration is low. In Chapter II, it has been shown the changes in rheological and thermal properties of GELL solutions caused by the addition of monovalent or divalent cations. It was suggested that cations shield the electrostatic repulsion between carboxyl groups in GELL molecules, so that the formation of thermally stable junction zones by the hydrogen bonds between GELL molecules were promoted. Although sugars can stabilise the junction zones of GELL gels, the stabilisation by addition of sugars seems to be essentially different from that by the addition of cations; cations stabilise the junction zones by the electrostatic interaction, while sugars stabilise these by newly created hydrogen bonds.

It is noteworthy that in the presence of any sugar of fairly low concentration, both T_s and T_m slightly shifted to lower temperatures (Figure III-12) and ΔH significantly decreased (Figure III-13) in DSC measurements, in contrast, G' (Figure III-10) and ΔG (Figure III-7) significantly increased in the presence of sugar of the same amount in the rheological measurement. However, further addition of sugar shifted T_s and T_m to higher temperatures and increased ΔH as well as elastic modulus. The increase in T_m, T_s and ΔH is caused by the formation of thermally stable junction zones. The increase of elastic modulus for gellan gum gels by the addition of sugar can be understood by the increase of the number of elastically active network chains or the number of junction zones estimated by a modified theory of rubber elasticity.

Oakenfull (1984) has proposed a method for using measurements of shear modulus to estimate the size of junction zones in noncovalently cross-linked gels. Oakenfull and Scott (1986) have reported that although sugars could stabilise gelatin gels and increase the formation of junction zones, calculations from shear modulus data showed that in the presence of sugar, the junction zones became smaller but at the same time more numerous, which resulted in a more extensive gel network and consequently greater rigidity of gel.

It has been suggested that the gel-sol transition of thermo-reversible gels is explained theoretically using zipper model which has been used for explaining the helix-coil transition and the melting of deoxyribonucleic acid (DNA) (Nishinari et al., 1990). As a model for structures of junction zones, it could be assumed such junction zones as the association of molecular zippers standing for a rigid ordered molecular structure such as helices or extended molecules. The molecular forces which make these helices or rods aggregate are generally believed to be secondary forces such as hydrogen bonds rather than covalent bonds because the disruption of the covalent bond needs much higher energy than experimentally observed values. It could, therefore, be simulated the dissolution of gels as an opening process of molecular zippers, and treated a molecular zipper of N parallel links that can be opened from both ends (Figure III-15). When the links 1, 2, ····, p are all open, the energy required to open the p +1 st link is assumed to be ε. It is supposed that each open link can assume G orientations, i.e., the open state of a link is G-fold degenerate, corresponding to the rotational freedom of a link. According to this treatment, the heat capacity C of such a system consisting of $\mathcal{N}$ zippers is written as follows:

$$C = \mathcal{N}\left(\log\frac{G}{x}\right)^2\left[\frac{2x}{(1-x)^2} + \frac{N(N+1)x^N\{-x^{N+1}+(N+1)x-N\}}{\{1-(N+1)x^N+N x^{N+1}\}^2}\right] \qquad (1)$$

where k is the Boltzmann constant, G is the degree of rotational freedom of a link, $x=G\exp(-\varepsilon/\tau)$, where τ is the product of k and the absolute temperature.

In terms of a zipper model approach, it has been suggested that the thermal stability of each junction zones depends on the number of parallel links N which forms a single zipper (junction zone) as well as on the bonding energy ε and the degree of rotational freedom G. As described previously, in all the cases of agarose (Watase and Nishinari, 1990; Watase et al., 1992; Nishinari et al., 1992), κ-carrageenan (Nishinari et al., 1990; Nishinari and Watase, 1992) and gelatin gels (Nishinari et al., 1992), the number of zippers $\mathcal{N}$ may increase whereas the number of parallel links N should decrease with increasing concentration of sugars, because the number of parallel links N may be proportional to the number average molecular weight of the junction zones which was determined by the rheological analysis (Oakenfull, 1984). The elastic moduli for gellan gum solutions increased by the addition of sugars indicating the increase of the number of elastically active network chains, however, the number of parallel links N may decrease with increasing concentration of added sugars, as in the case of agarose (Watase and Nishinari, 1990; Watase et al., 1992; Nishinari et al., 1992), κ-carrageenan (Nishinari et al., 1992) or gelatin (Nishinari et al., 1992). Therefore, the storage modulus G' as a function of the number of zippers $\mathcal{N}$ was obtained from the curve fitting assuming that the number of zippers $\mathcal{N}$ as well as shear modulus increase with increasing concentration of sugars, as shown in Figure III-16. It is clear that the number of parallel links N should decrease with increasing concentration of sugars, which is consistent with previous findings (Oakenfull and Scott, 1986; Nishinari et al., 1992) described above.

Figure III-17 shows the DSC heating curves for 1% gellan gum solutions with and without glucose (solid lines) and the best fitted calculated curves (dotted lines) using a zipper model approach. Here, the bonding energy ε is fixed as 2000k, which is the approximate value for hydrogen-bonding energy, and the number of parallel links N is assumed as 100 for a gellan gum solution without sugar. The values of N are taken as 60 for a solution with 0.05M glucose, 55 for a solution with 0.45M glucose, and 36 for a solution with 1.5M glucose, as is expected from Figure III-16. The number of zippers $\mathcal{N}$ per one gram gel and the rotational freedom of links G are determined from the curve fitting. As illustrated in this figure, in the presence of 0.05M glucose, the number of zippers $\mathcal{N}$ increased compared to that without sugars, however the number of parallel links N, which is proportional to the size of junction zones, decreased and the rotational freedom of link G increased. It, therefore, seems that the addition of sugar to GELL solutions increases the number of junction zones, however, the size of each zipper becomes small if the amount of sugar is not sufficient, so that the addition of sugar of fairly low concentration could increase the elastic moduli but decrease thermal stability. In other words, in the presence of sugar, the junction zones in GELL molecules become smaller, which should make junction zones thermodynamically weaker. As shown in Figure III-1~III-6, the experimental findings, that in the presence of sugar of fairly low concentration, the values of G" at higher temperature sides slightly decreased rather than those without sugar may be consistent to this interpretation. In the presence of 0.45M, the number of parallel links N further decreased, however, the number of zippers $\mathcal{N}$ significantly increased and then the rotational freedom of links G decreased. Therefore, with progressive addition of sugars, small but more numerous junction zones were formed, so that the numerous small junction zones could increase not only the elasticity but also thermal stability of gellan gum gels, which could be explained by the facts that both the elastic moduli and ΔH increased by the further addition of sugars. This result was in good agreement with the results in the case of agarose (Watase and Nishinari, 1990; Watase et al., 1992; Nishinari et al., 1992a), carrageenan (Nishinari et al., 1992a) or gelatin (Nishinari et al., 1992a). However, it was never observed in the previous studies that the thermal stability became weaker by the addition of sugars. This difference between previous studies and this study may be induced by the difference of concentration of polysaccharide. In the presence of sufficient sugars, the elastic moduli and thermal stabilities of junction zones for GELL gels markedly increased, however, the cooling and heating DSC curves for GELL solutions became broader by the addition of sufficient sugars (Figure III-11). Since the addition of sugars may create hydrogen bonds, the distribution of energy required to open the link and that of the rotational freedom G of a link will become broader. Similar behaviours were observed previously for agarose (Watase and Nishinari, 1990; Watase et al., 1992; Nishinari et al., 1992), carrageenan (Nishinari et al., 1992) or gelatin (Nishinari et al., 1992). Gekko (1987) has reported that the addition of sugar to κ-carrageenan raised the gelling temperature with an increase in their concentration, accompanying a decreased enthalpy of gelation. Moritaka et al.(1994) have reported that the storage modulus G' decreased by the addition of sugars. Whether this was due to the difference in the concentration range examined in the previous report (Nishinari et al., 1990) or due to other factors involving the effects of cations in the samples is not clear at the present stage and should be clarified in the future.

As described previously, the temperature dependence of elastic modulus for thermoreversible gels has been explained on the basis of a reel-chain model consisting junction zones and flexible chains connecting the junction zones at the temperature range lower than the gel-sol transition (Nishinari et al., 1985). According to this model, temperature dependence of elastic modulus may be explained in terms of bonding energy ε, the mean end-to-end distance r_m of chains which connect junction zones, and the ceiling number ν, i.e., the upper limit of the number of segments which can be liberated from junction zones just before the transition from gel-to-sol occurs. For a smaller value of ε, r_m or ν, the elastic modulus decreases monotonically with increasing temperature. For intermediate values of ε, r_m or ν, the elastic modulus increases up to a certain temperature and then decreases with increasing temperature. For large values of ε, r_m or ν, the elastic modulus increases monotonically with increasing temperature. It is well known that the temperature dependence of G' for rubber which has large ε shows the monotone increasing behaviour with increasing temperature while the behaviour for carrageenan and gelatin and many other thermoreversible gels which have small ε shows the monotone decreasing behaviour with increasing temperature (Nishinari and Watase, 1992; Watase and Nishinari, 1990; Nishinari et al., 1992). It has been reported that the temperature dependence of E' for agarose gels except for dilute gels shows a maximum at a certain temperature (Nishinari and Watase, 1992; Watase and Nishinari, 1990; Nishinari et al., 1992). The difference between the temperature dependence of elastic moduli for carrageenan and agarose seems probably to be caused by the fact that the bonding energy ε for agarose gels was significantly larger than that for carrageenan gels. As shown in Figure III-1~III-6, the temperature dependence of elastic moduli for gellan gum solutions in the presence of sugar at lower concentrations was similar to that for carrageenan gels which showed the monotone decreasing behaviour with increasing temperature, however, that for gellan gum solutions in the presence of sufficient sugar was close to the behaviour for agarose gels which showed a maximum at a certain temperature. Since the bonding energy ε does not seem to change with increasing concentration of sugar, the ceiling number ν must increase with increasing concentration of sucrose. This may be caused by the decrease in the rotational freedom of parallel links which constitute a molecular zipper, as described in Figure III-16. In other words, as described in Figure III-16, the number of junction zones increases, which in turn makes the end-to-end distance of flexible chains connecting junction zones shorter with increasing concentration of sugar.

The elastic moduli and thermal stabilities of junction zones increased by the addition of sugars in the order of effectiveness: sucrose > glucose > mannose. However, hydrated water by sugar is less important at low concentration of sugar, as mentioned above. The different effects of glucose and sucrose on G' [Figure III-10(a),(b)] could be explained by the difference in changing the water structure. Since sucrose has a larger number of e-OH groups in their molecules than glucose, it seems that sucrose immobilizes much more free water than glucose. Therefore, with progressive addition of sucrose, the formation of thermally stable junction zones were promoted by newly created hydrogen bonds, however, the excessive addition of sucrose may reduce the amount of free water necessary to form junction zones, which induced the decrease of elastic moduli. Mannose, which has only 3.3 e-OH groups, hardly affects the water structure (Uedaira and Ishimura, 1989; Uedaira and Ohsaka, 1991), thus, shows the least effect on rheological properties of gellan gum. The results of T_s, T_m or ΔH_m as a function of KGM concentration indicated that KGM little influenced the thermal stability of GELL solutions in comparison with sugars, however, KGM with relatively lower molecular weight significantly increased ΔG observed by the rheological measurement. Thus, in the presence of KGM with relatively lower molecular weight, the effective concentration of GELL may increase by immobilising water molecules in GELL solutions (only second possibility in sugars as mentioned above), so that KGM with relatively low molecular weight could indirectly promote the helix-coil transition of GELL molecules. Therefore, the effect of the KGM with relatively lower molecular weight to the rheological and thermal properties of GELL solution seems to be essentially different from that of sugar, which stabilizes junction zones of GELL molecules by forming hydrogen bonds. KGM with relatively higher molecular weight may inhibit helix-coil transition in GELL molecules, moreover, it may hinder further aggregation of GELL helices, because KGM with relative higher molecular weight decreased both ΔG observed by rheological measurement [Figure III-9(a),(b)] and ΔH_m observed by DSC.

The effects of sugar does not seem to be a direct result of changes caused by intermolecular binding between hydroxyl groups in gellan gum and in sugars stabilising gellan gum helices. Rather, sugars indirectly influenced the interaction between gellan gum and water as solvent, due to hydration in gellan gum gels in the presence of sugars. Therefore, it was concluded that sugar will increase the number of elastically active network chains by forming hydrogen bonds through the water molecules in gellan gum solutions, so that the elastic moduli and thermal stability of gellan gum solutions gradually increase with increasing concentration of sugar.

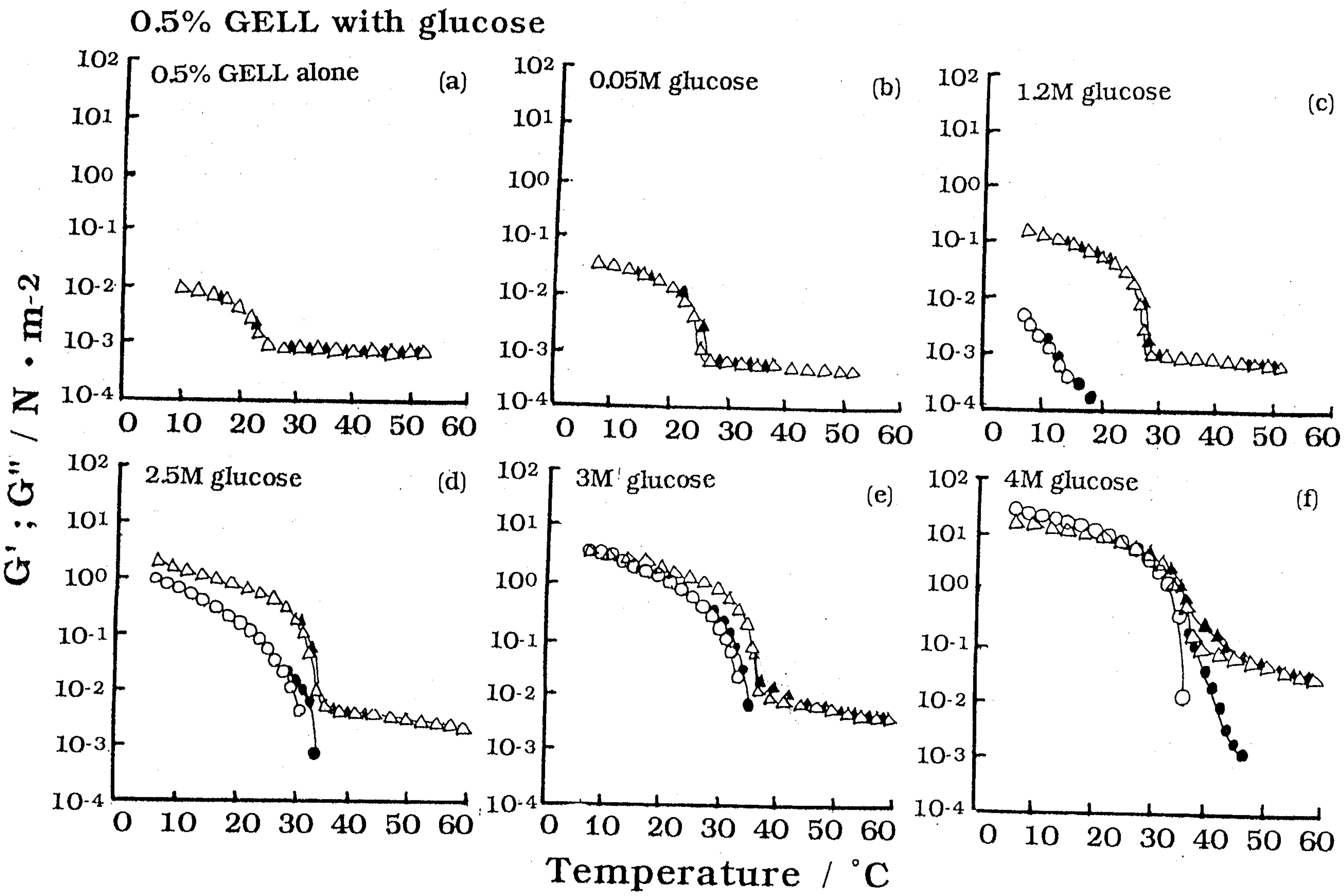

Fig. III-1 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a 0.5% gellan gum solution containing glucose of various concentrations.
(○)G', (△)G", cooling; (●)G', (▲)G", heating;
cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s;
Glucose conc., 0M(a), 0.5M(b), 2M(c), 2.5M(d), 3M(e), 4M(f).

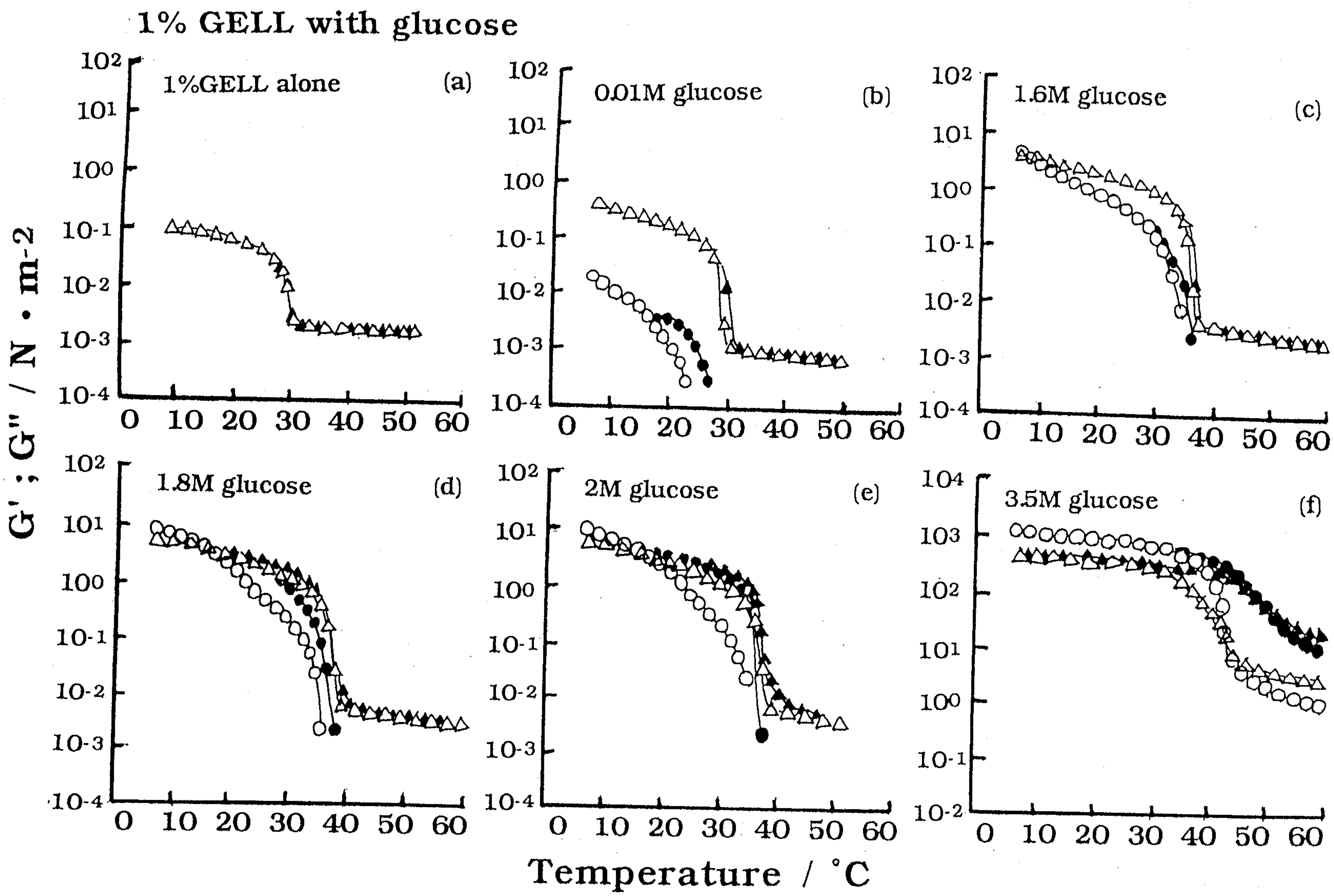

Fig. III-2 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a 1.0% gellan gum solution containing glucose of various concentrations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5°C/min; Freq.ω=0.1rad/s; Glucose conc., 0M(a), 0.01M(b), 1.6M(c), 1.8M(d), 3M(e), 3.5M(f).

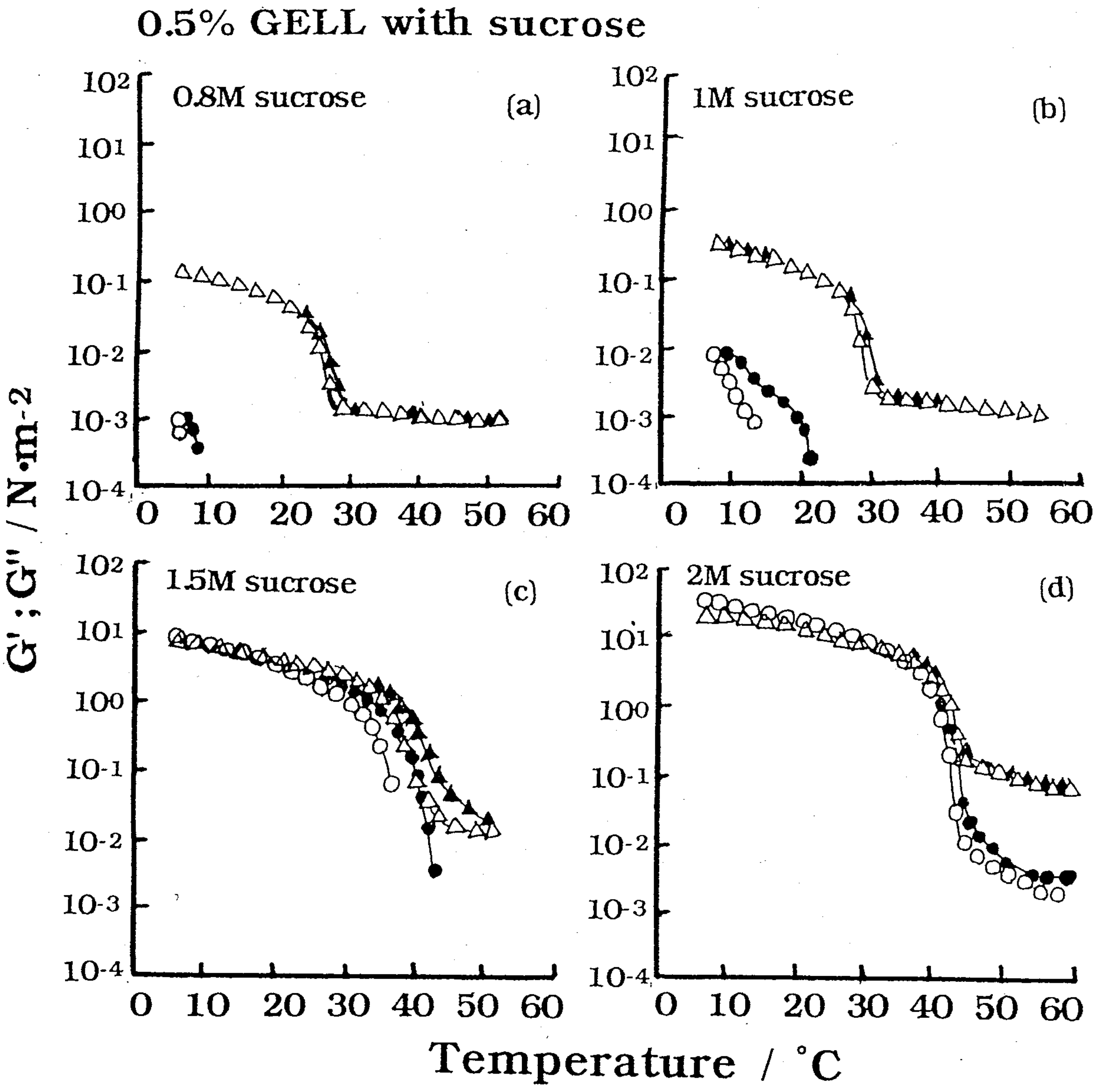

Fig. III-3 Temperature dependence of storage modulus G' and loss modulus G'' during cooling and heating process for a 0.5% gellan gum solution containing sucrose of various concentrations.
(○)G', (△)G'', cooling; (●)G', (▲)G'', heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s; Sucrose conc., 0.5M(a), 1M(b), 1.5M(c), 2M(d)

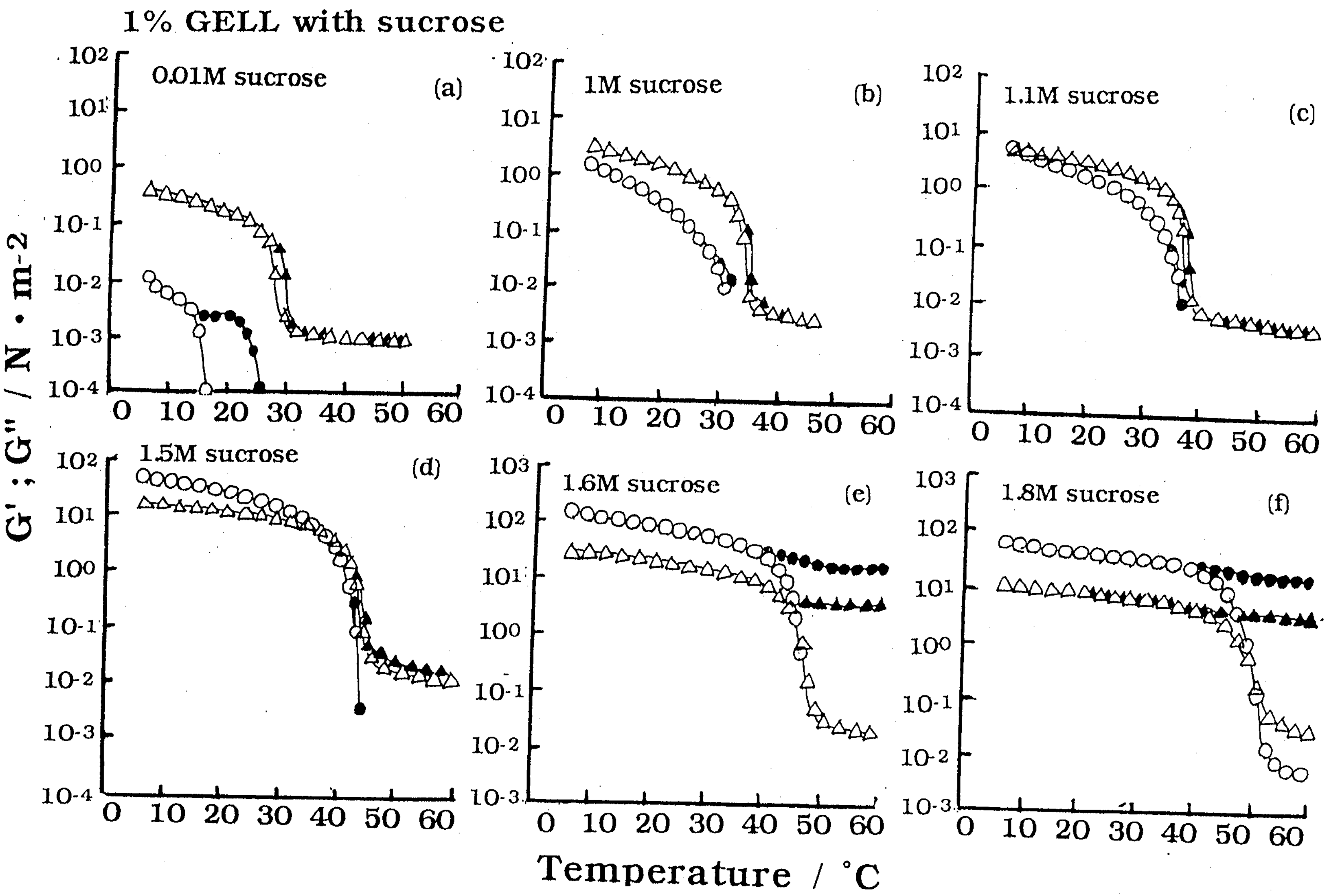

Fig. III-4 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a 1.0% gellan gum solution containing sucrose of various concentrations.
(○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s; Sucrose conc., 0.01M(a), 1M(b), 1.1M(c), 1.5M(d), 1.6M(e), 1.8M(f).

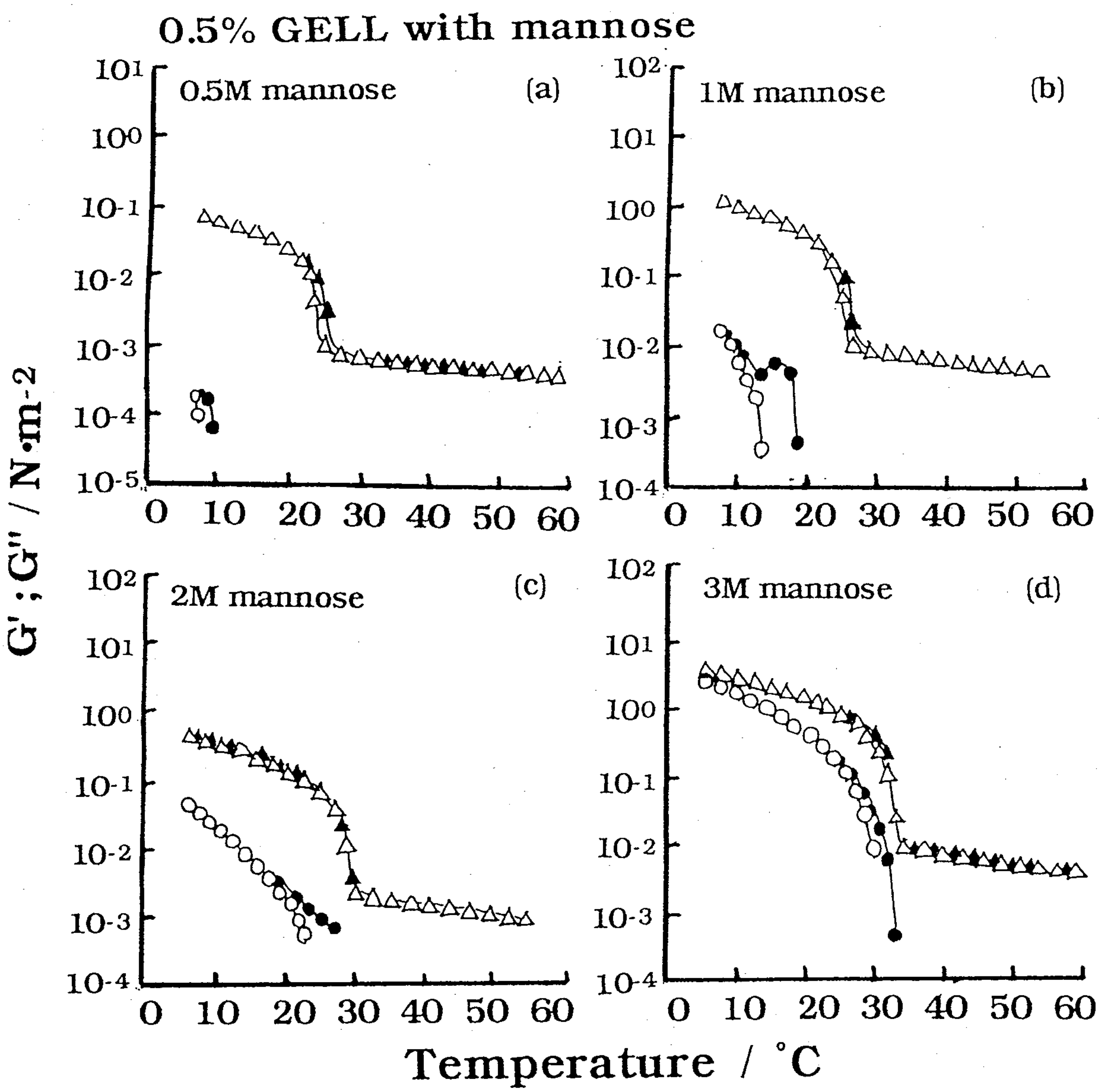

Fig. III-5 Temperature dependence of storage modulus G' and loss modulus G'' during cooling and heating process for a 0.5% gellan gum solution containing mannose of various concentrations. (○)G', (△)G'', cooling; (●)G', (▲)G'', heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s; Mannose conc., 0.5M(a), 1M(b), 2M(c), 3M(d)

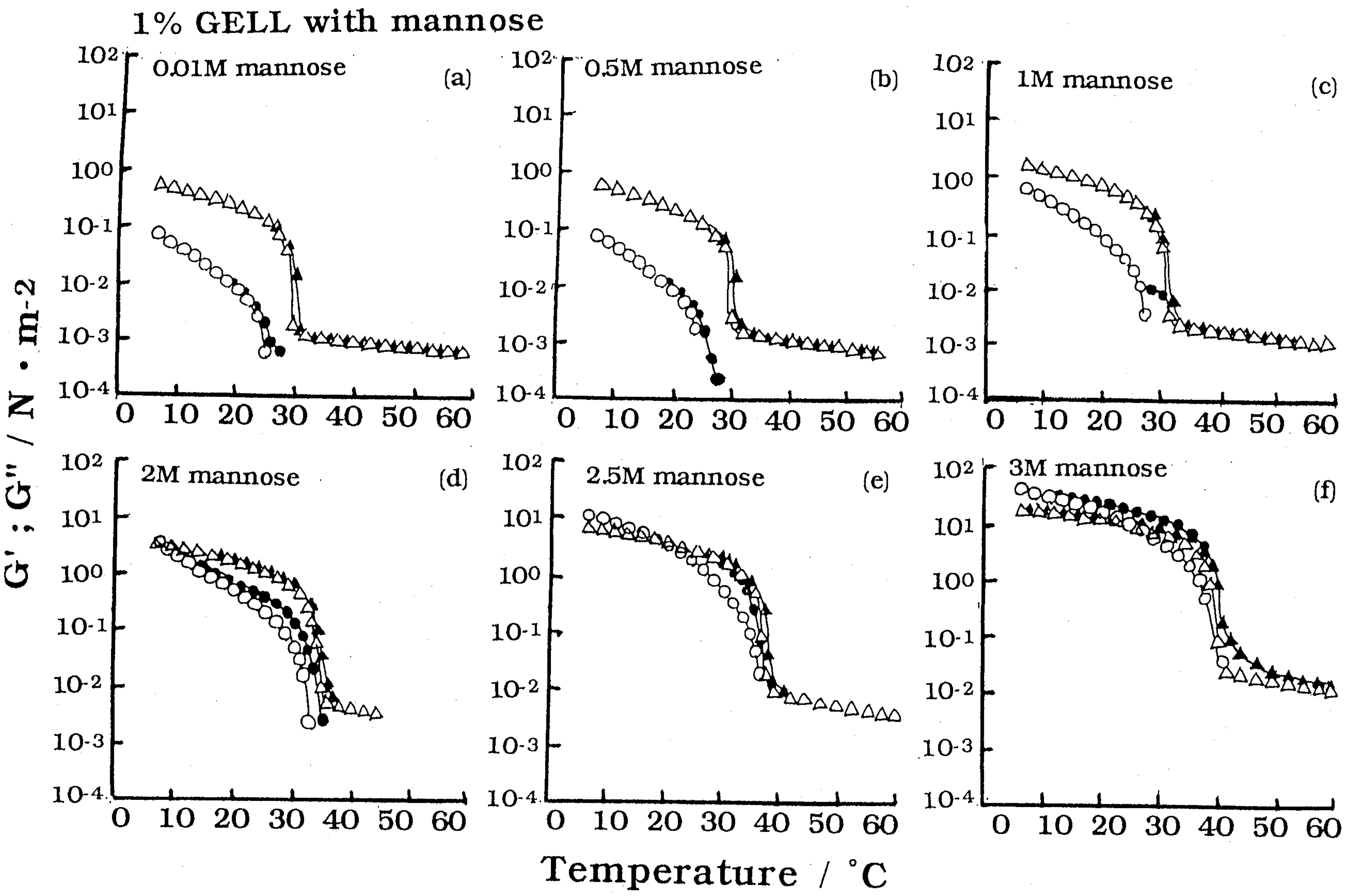

Fig. III-6 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a 1.0% gellan gum solution containing mannose of various concentrations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate:0.5℃/min; Freq.ω=0.1rad/s; Mannose conc., 0.01M(a), 0.5M(b), 1M(c), 2M(d), 2.5M(e), 3M(f).

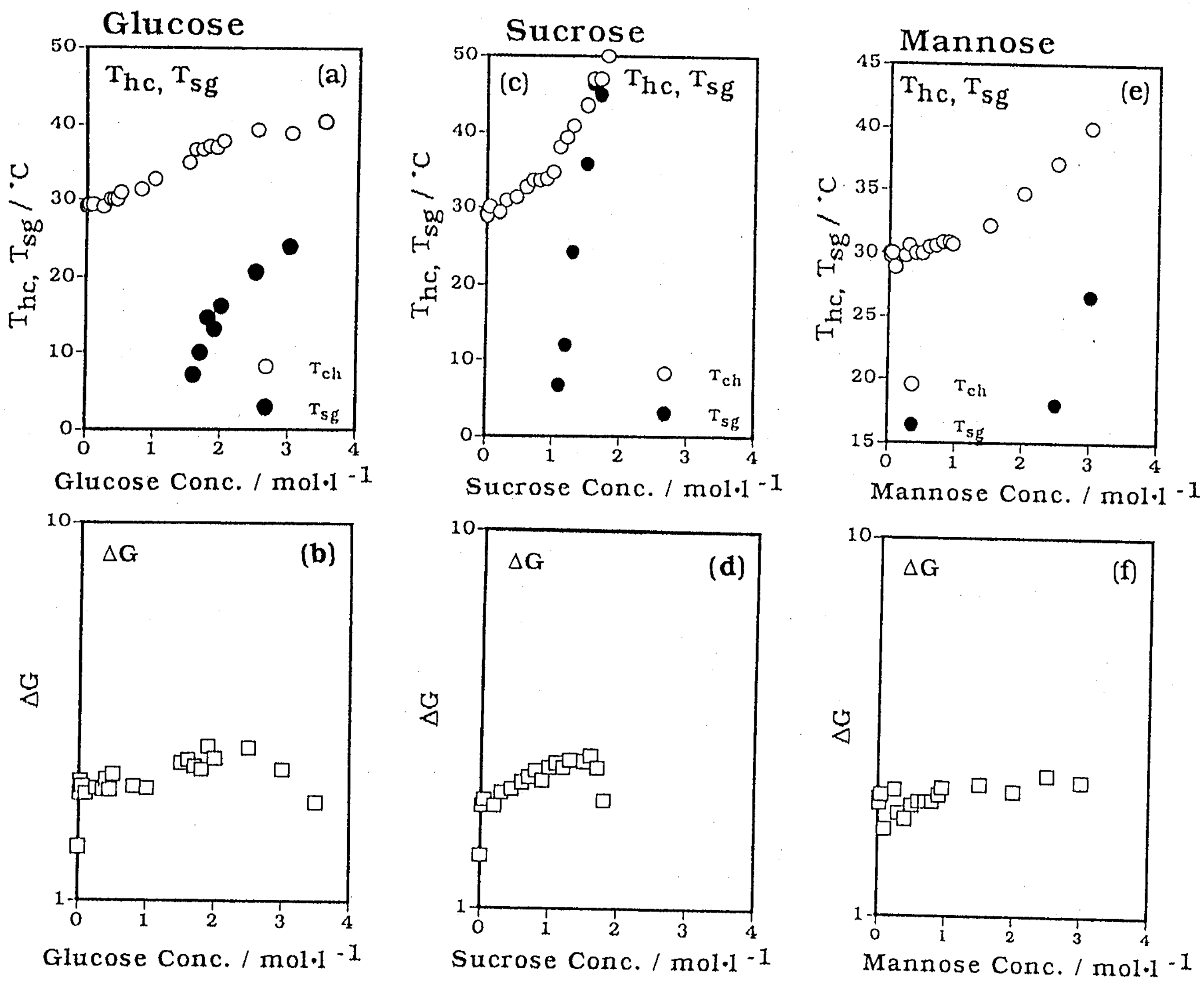

Fig. III-7 Helix-coil transition temperature T_{hc}, the sol-gel transition temperature T_{sg} and the relaxational strength ΔG for 1% gellan gum solutions as a function of the added concentration of glucose, sucrose and mannose. (a) T_{hc} and T_{sg}, (b) ΔG, glucose; (c) T_{hc} and T_{sg}, (d) ΔG, sucrose; (e) T_{hc} and T_{sg}, (f) ΔG, mannose.

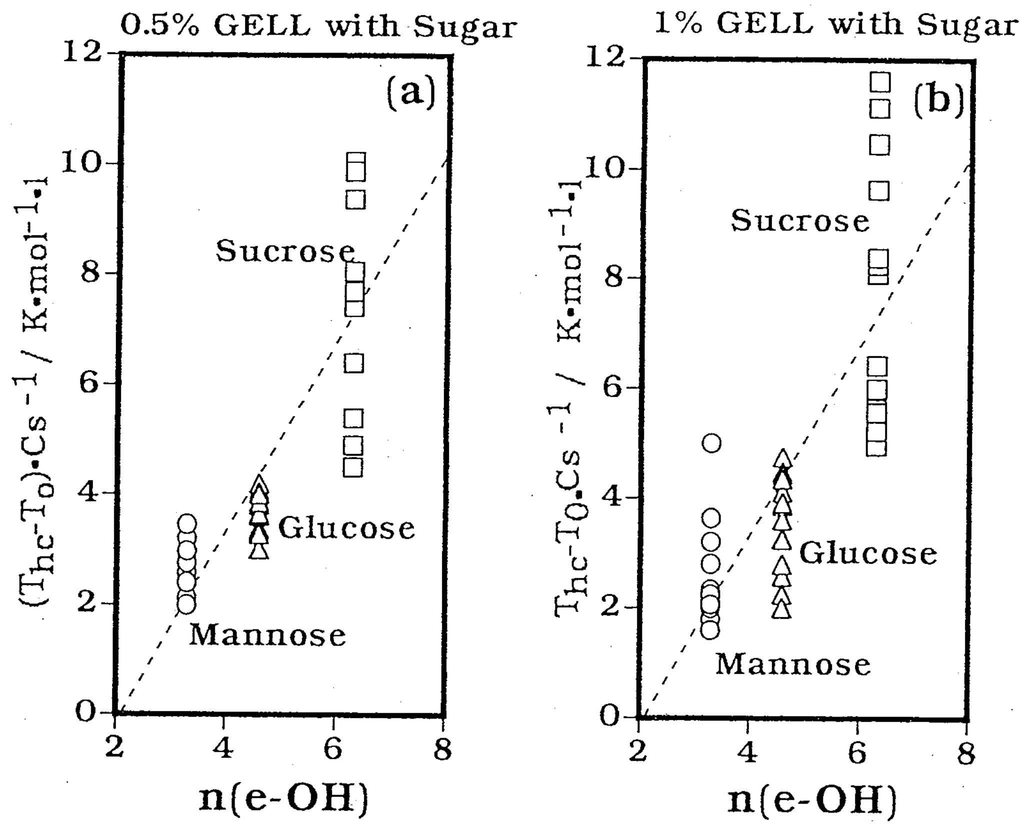

Fig. III-8 The relation between the increment of the helix-coil transition temperature ΔT_{hc} and the number of equatorially attached OH groups in a sugar added. Gellan conc., 0.5%(a), 1.0%(b); (△)glucose, (□)sucrose, (○)mannose.

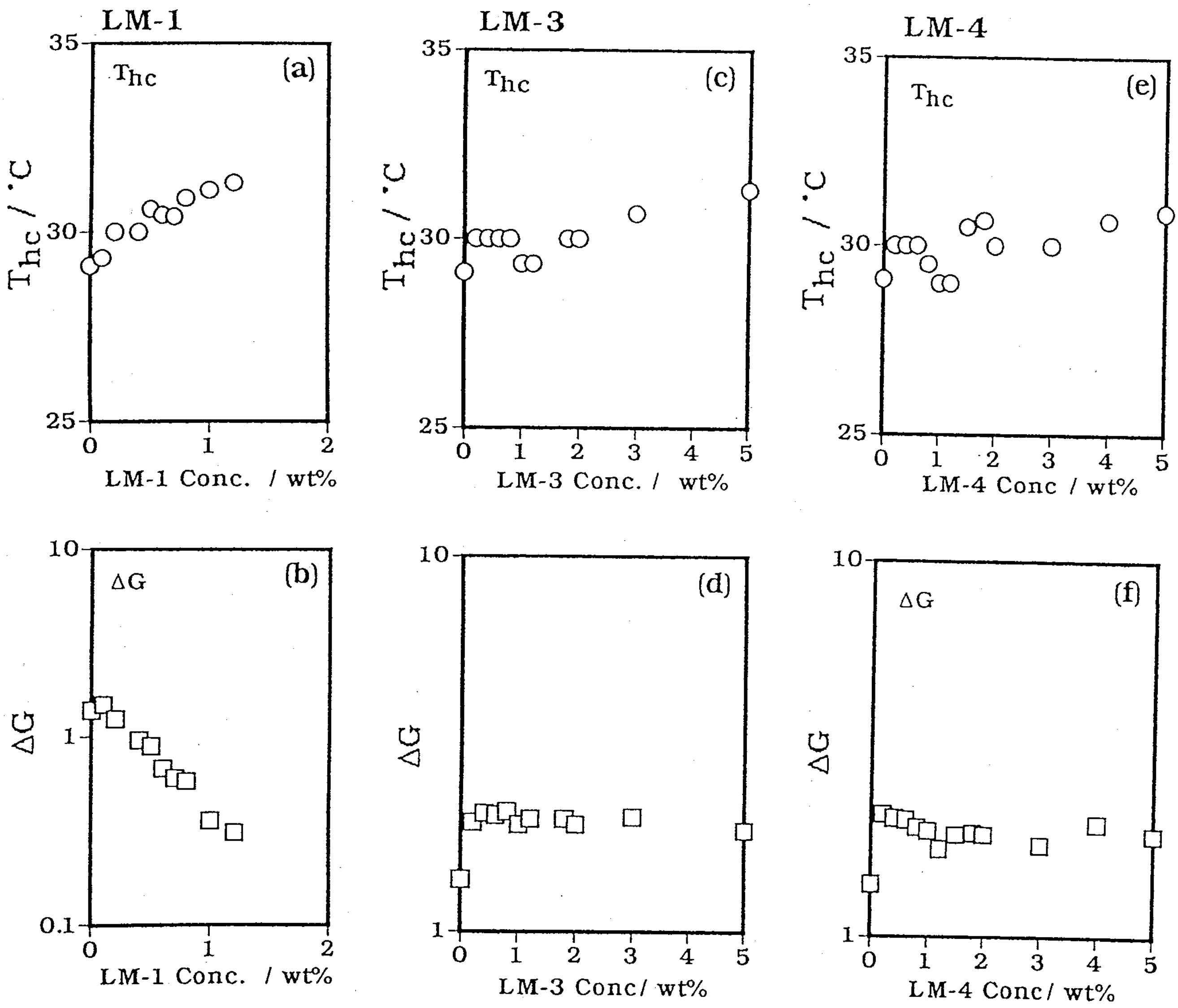

Fig. III-9 Helix-coil transition temperature T_{hc}, and the relaxational strength ΔG for 1% gellan gum solutions as a function of the added concentration of LM-1, LM-3 and LM-4. (a)T_{hc}, (b)ΔG, LM-1; (c)T_{hc}, (d)ΔG, LM-3; (e)T_{hc}, (f)ΔG, LM-4.

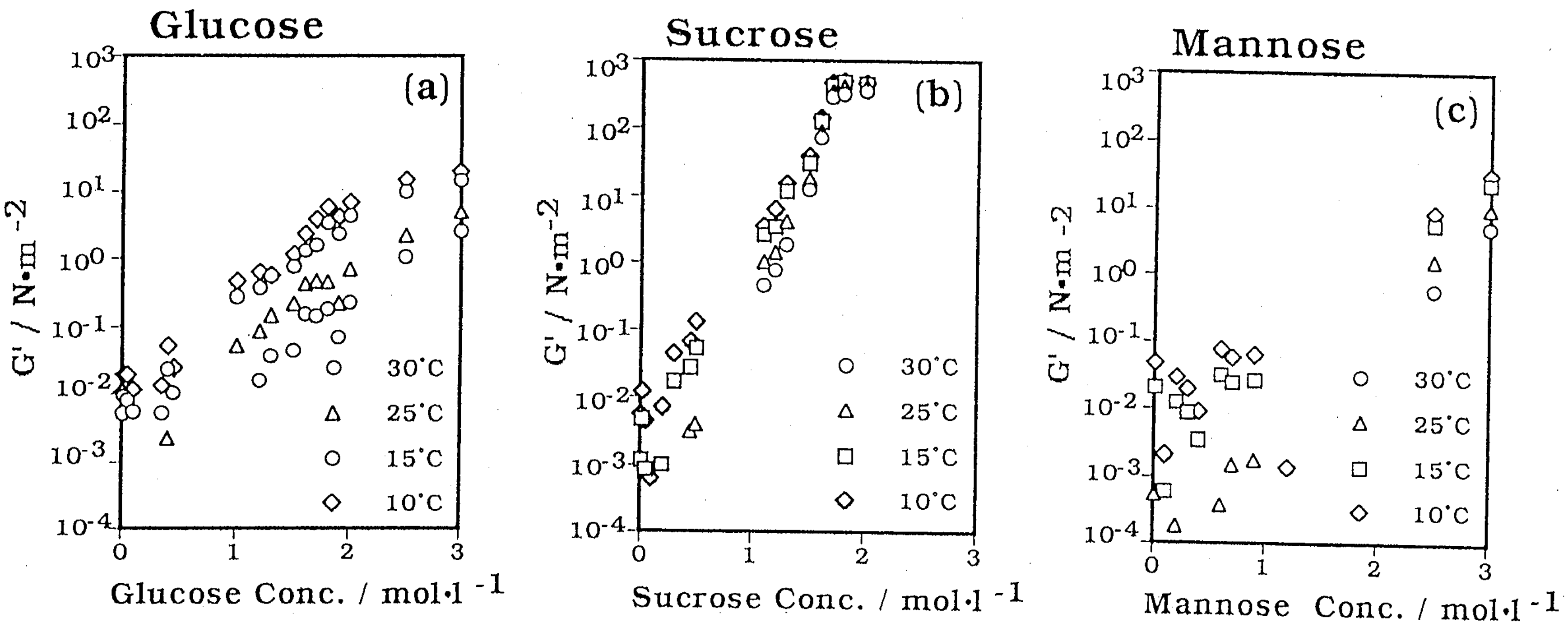

Fig. III-10 Storage modulus G' of gellan gum solution as a function of concentration of glucose, sucrose or mannose at various temperatures.

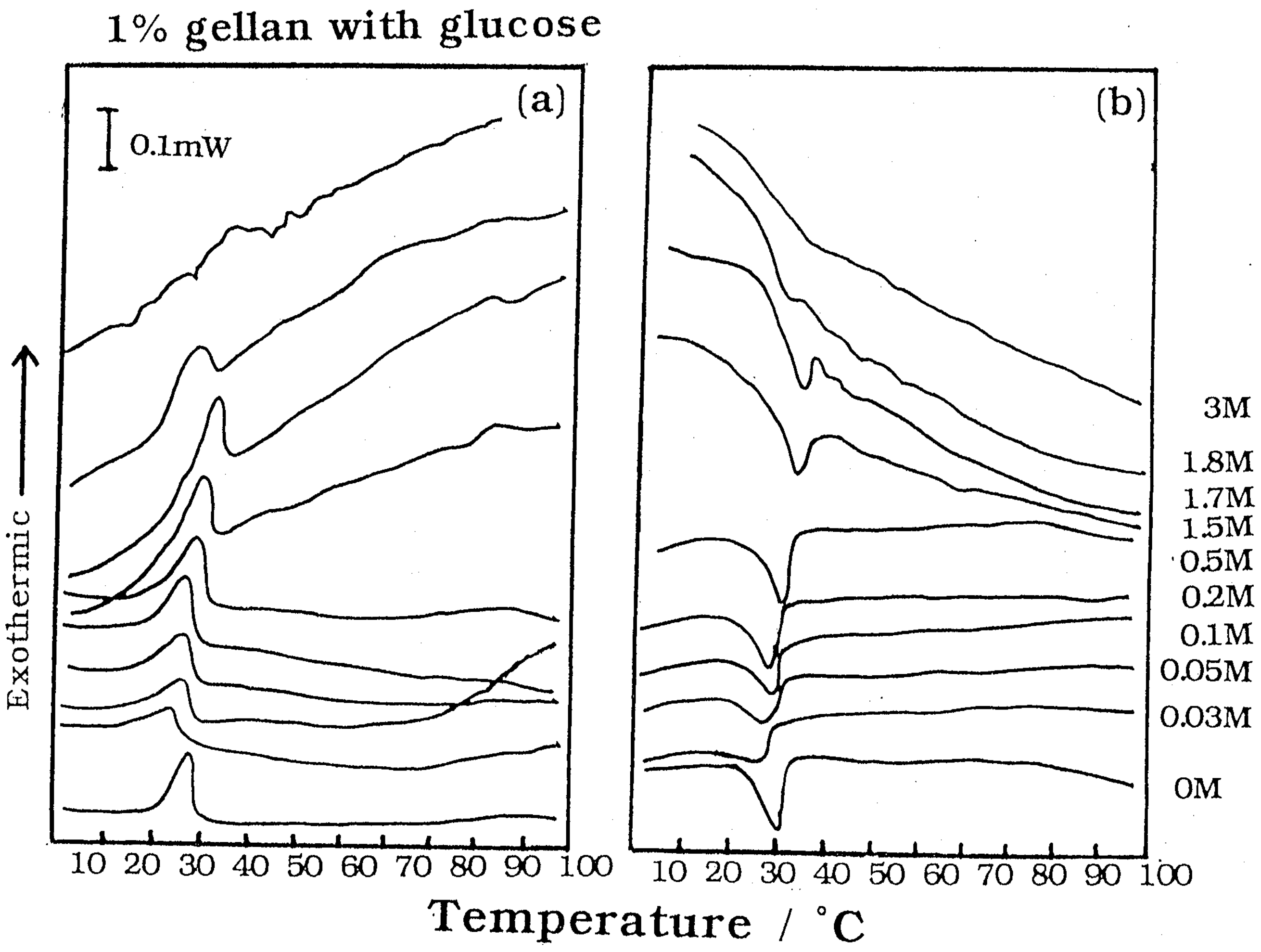

Fig. III-11 Cooling (a) and heating (b) DSC curves for 1% gellan gum solutions containing glucose of various concentration. Figures beside each curve represent the concentration of gellan gum. Cooling and heating rate: 0.5℃/min.

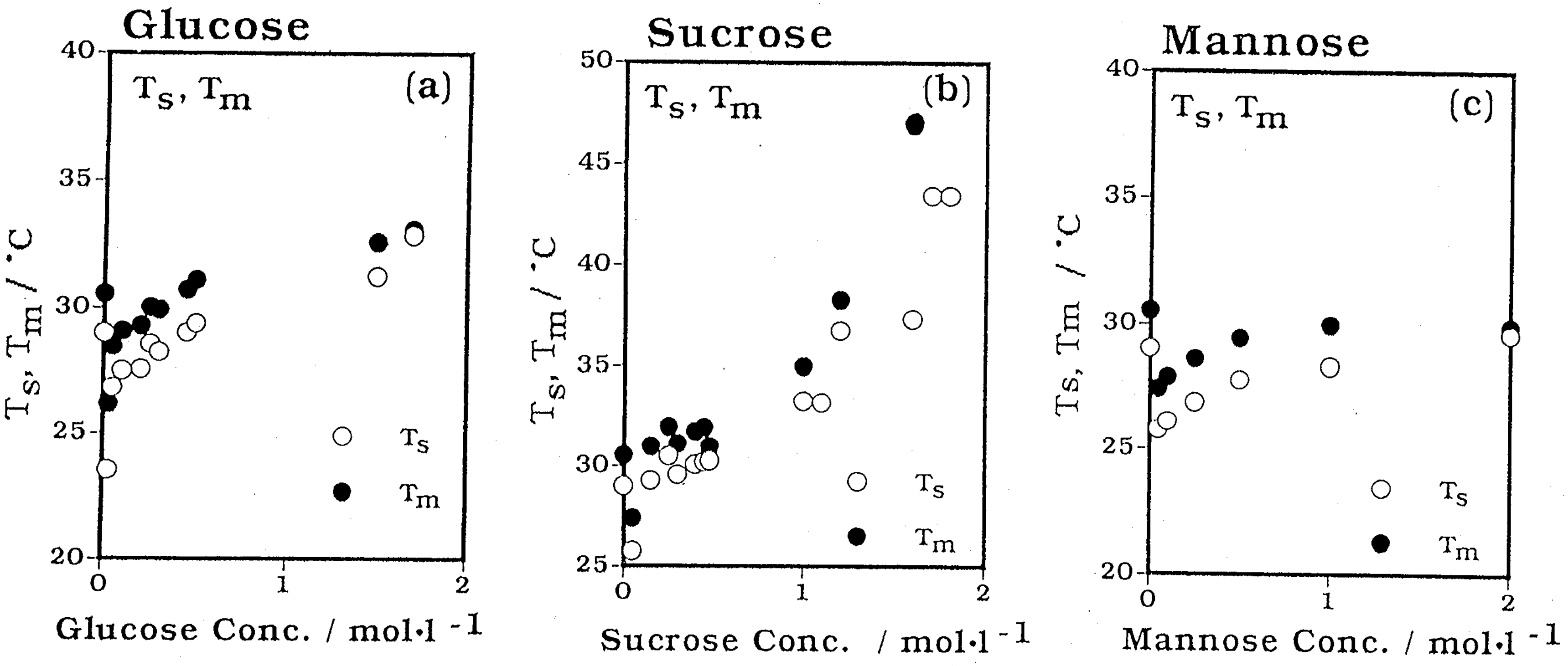

Fig. III-12 The exothermic and endothermic peak temperature, T_s and T_m, for 1% gellan gum solutions as a function of the added glucose(a), sucrose(b) or mannose(c).

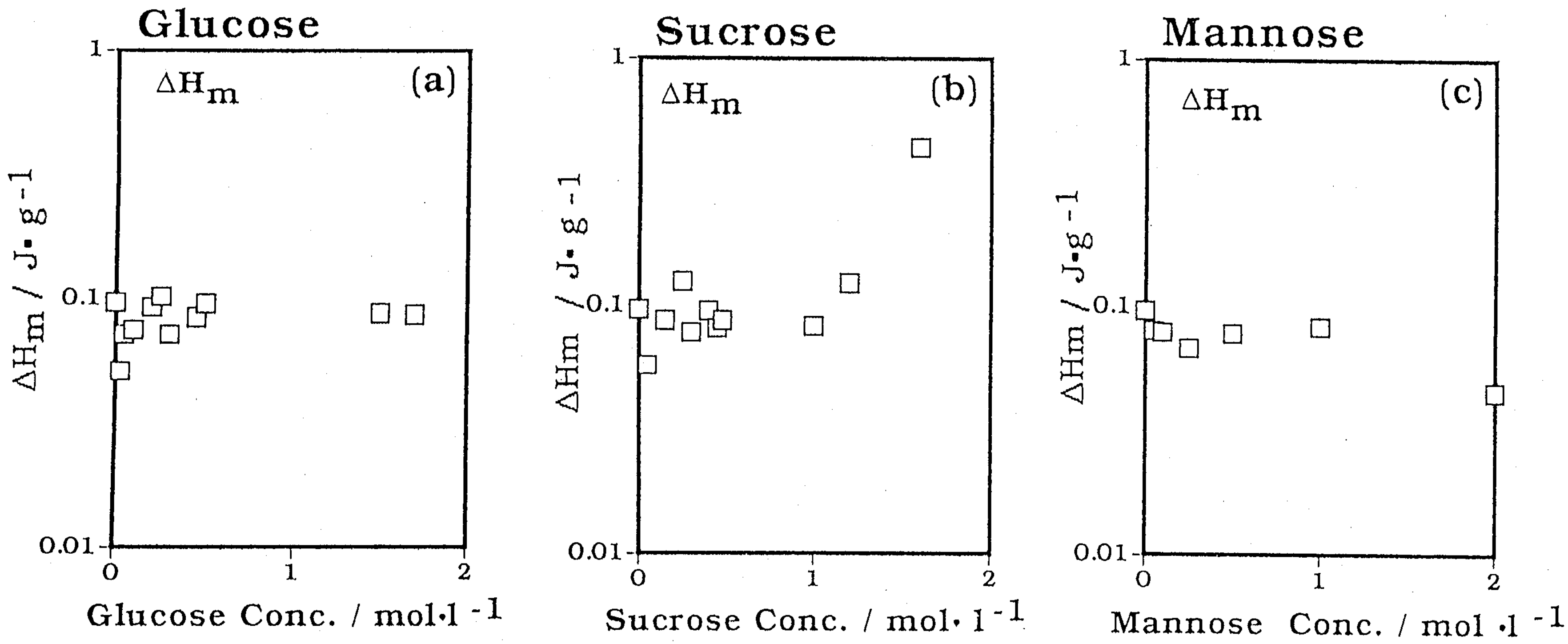

Fig. III-13 The endothermic enthalpies ΔH_m for 1% gellan gum solutions as a function of the added glucose(a), sucrose(b) or mannose(c).

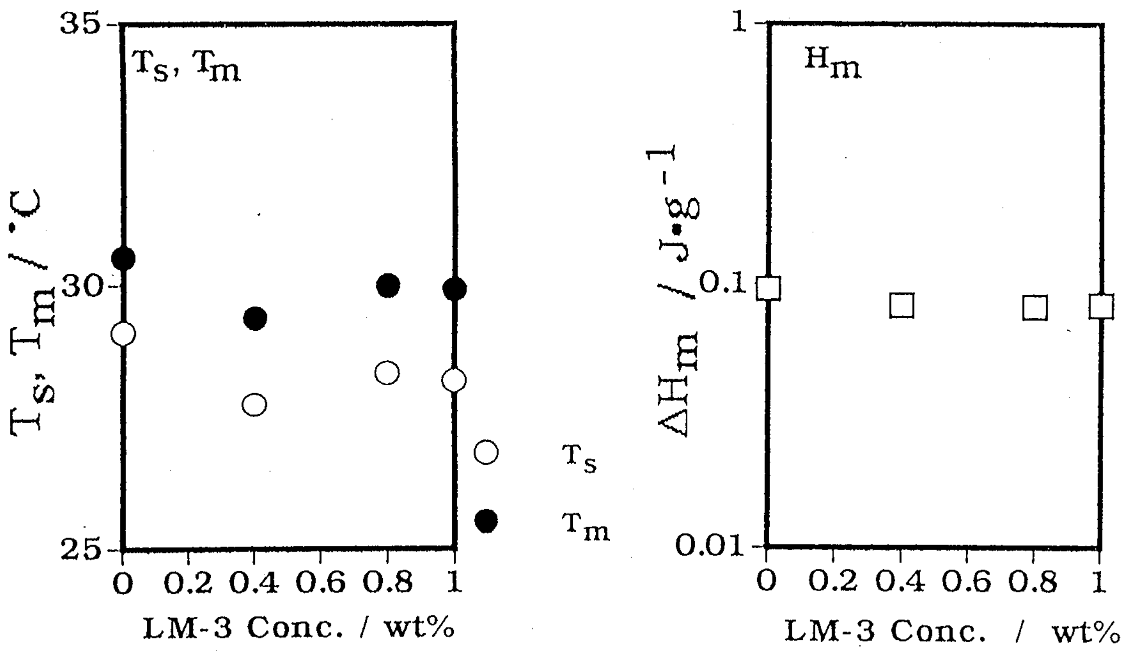

Fig. III-14 The exothermic and endothermic peak temperature, T_s and T_m, and the endothermic enthalpies Hm for 1% gellan gum solutions as a function of the added LM-3.

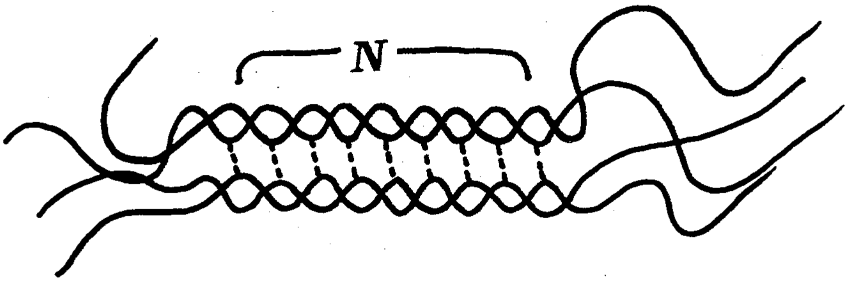

Fig. III-15 A single zipper with *N* links. Links can be opened from both ends. If the links 1, 2, ·····, p are all open, the energy required to open the p+1st link is ε. Helices may not necessarily be double helices; they may be single or triple helices. Here, double helices represent the association of somewhat ordered structure symbolically. (Nishinari et al., 1990c)

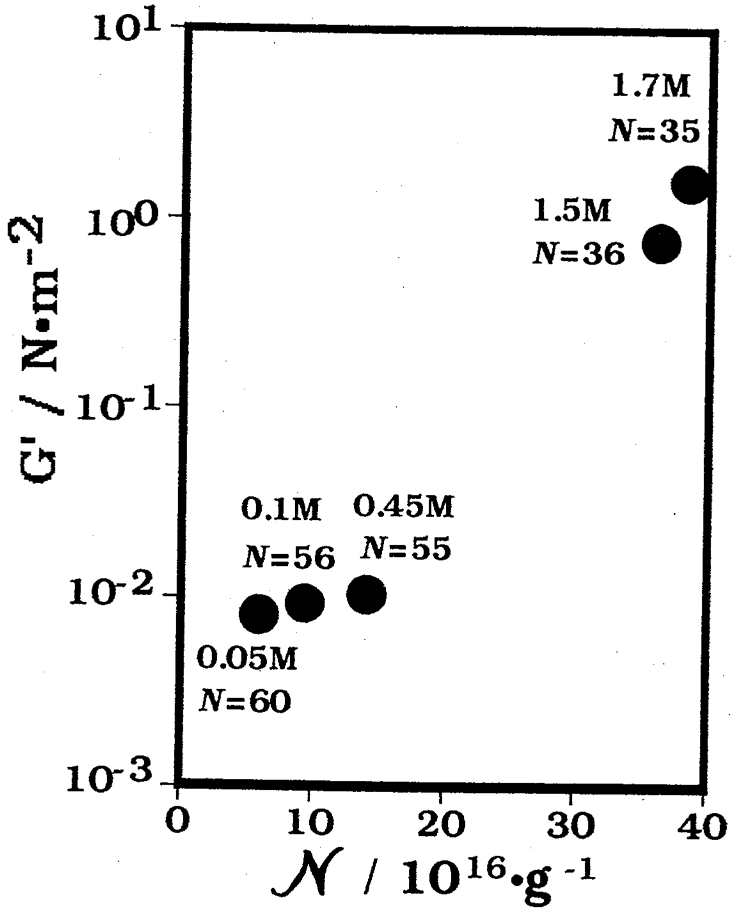

Fig. III-16 Storage modulus G' in the rheological measurement as a function of the number of zippers $\mathcal{N}$ calculated using zipper model approach. N; the number of parallel links assumed from the best fitted curve.

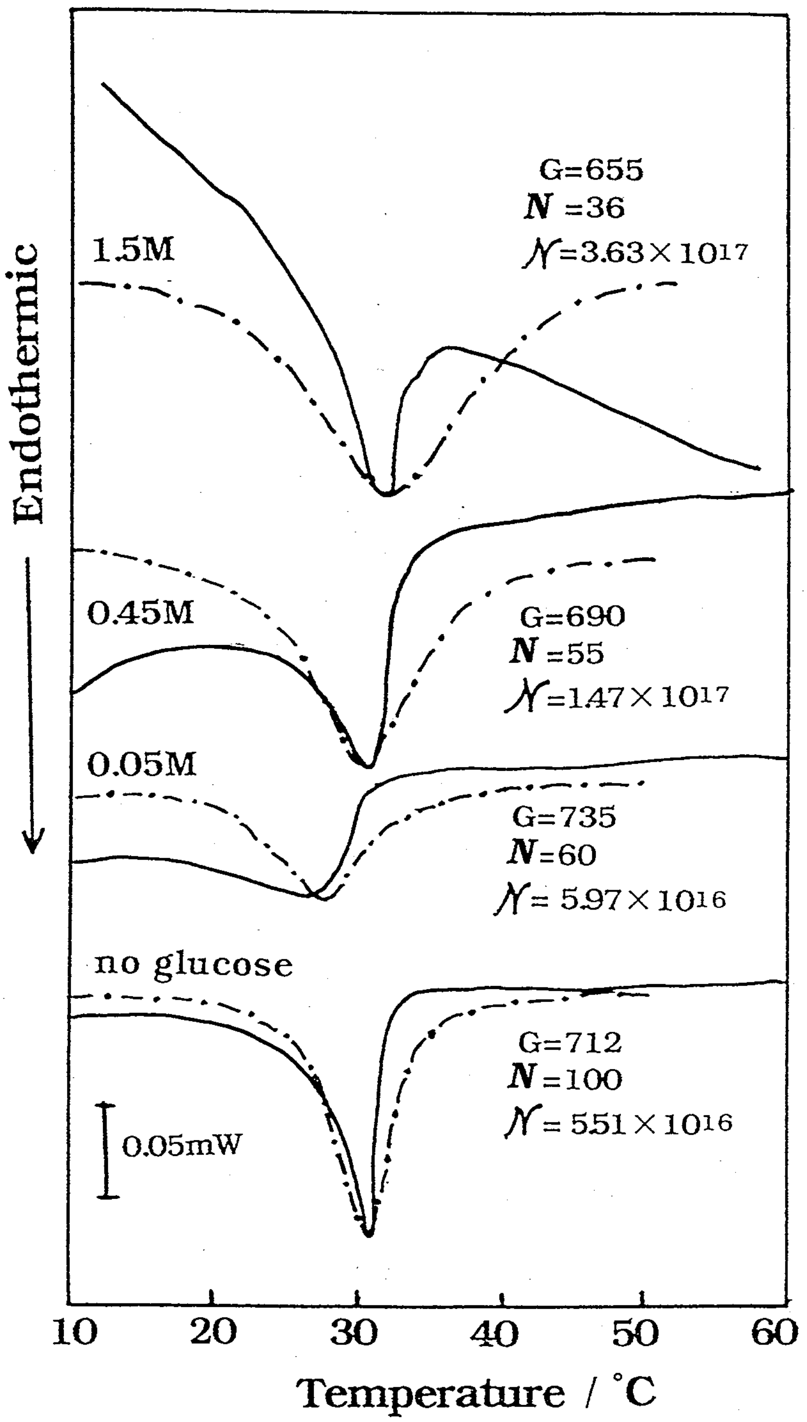

Fig. III-17 Heating DSC curves of 1% gellan gum solutions with and without glucose (solid lines) and the best fitted calculated curves (dotted lines) using a zipper model approach. Heating rate; 0.5°C /min. The energy ε is fixed as 2000k. The number of parallel links N is assumed as 100 for a 1% gellan gum solution without glucose, and is assumed as each value for solutions with glucose of various concentrations, which is determined from Figure 15. The number of zippers $\mathcal{N}$ per gram and the rotational freedom of links G are determined from the curve fitting. These values are shown beside each curve.

IV-1. "Interaction between Helix-Forming Polysaccharide and Galactomannan or Konjac Glucomannan"

Many polysaccharides have found widespread applications in the food industry because they form viscous solutions and gels which are extremely useful in the texturing of foodstuffs. Often the mixed systems of two polysaccharides are employed to manipulate rheological properties, since the properties of mixtures are superior to those of either component alone. For example, such mixed systems give stronger gels than either of the individual polymers or give the same gel strength at a lower total concentration, which provides effective savings in production costs (E.R.Morris, 1990b). Extensive studies on gelation mechanism of such mixtures have been carried out since the 1970s, and still gives rise to considerable controversy. Cairns et al. (1987) have classified the mixture gels into four different types and these are illustrated in Figure IV-1-1. Structure(a) shows the case with only one of the polysaccharides contributes to the gel network and the second polymer is simply entrapped within the matrix. If both polysaccharides contribute to the network, the following three cases are possible: (b) separate polymer networks which interlace and form interpenetrating networks; (c) a phase-separated network; (d) one polysaccharide binds to the other to form a coupled network defined by specific junction zones. The most familiar and widely explored mixed gels of food hydrocolloids are probably those involving galactomannans in combination with κ-carrageenan, furcellaran, agar or xanthan, which forms ordered structures. The addition of galactomannan to these polysaccharides (like agarose or κ-carrageenan) is known to improve mechanical properties and reduce brittleness or syneresis of the polysaccharide gel (V.J.Morris, 1986). The synergistic effects between galactomannan and these polysaccharides lead to gels with increased elasticity and strength under conditions at which the pure components would not gel. More spectacularly, galactomannan and xanthan also form gels despite the fact that they neither form a gel independently at neutral pH. The mechanism of gelation of these mixtures has been the subject of much interest since early studies (Dea and Morrison, 1975; Dea, 1979) and is a matter of debate and several models have been proposed (Cairns et al., 1986; Cairns et al., 1987;

Brownsey et al., 1988; Tako and Nakamura, 1986; Fernandes at al., 1991 and 1992; Rochas et al., 1990; Williams at al., 1991, 1993 and 1994; Cheetham and Mashimba, 1988 and 1991; Shatwell at al., 1991; Turquois et al., 1992; Kohyama et al., 1993). It was first found that xanthan gum and locust bean gum formed thermoreversible gels on mixing, although there was no evidence of gelation in xanthan-guar gum mixtures (Ohashi and Matsunaga, 1965). The original model for gelation of the mixture postulated interaction between the helical structure of the xanthan, carrageenan or agarose and the unsubstituted mannan regions, the so-called "smooth region" of galactomannan chains, as shown in Figure IV-1-2 (Dea and Morrison, 1975; Dea, 1979). X-ray diffraction studies, in contrast, did not provide evidence for a specific interaction or evidence of mannan/κ-carrageenan complexation, and it was suggested that the most likely model for such gels consists of a galactomannan solution contained within a carrageenan network (Cairns et al., 1986 and 1987; Brownsey et al., 1988) as shown in Figure IV-1-3. These workers also suggested that it was possible that other mannan chains adsorbed onto large aggregates of helices and Williams et al.(1993), and Piculell et al.(1994) provided further support for this. V.J.Morris (1986), E.R.Morris (1990b), and Williams (1994) has reviewed the mechanism of gel formation and characterization of physical properties in mixed polysaccharide gels.

Konjac glucomannan (KGM) is a glucomannan obtained from the tuber of Amorphophallus konjac K.Koch (Okimasu, 1975; Nishinari et al., 1992b), and forms a thermally stable gel on addition of alkaline coagulant. KGM is solubilised by a very low level of acetate substitution (about 1 acetyl group per 19 sugars), and removal of these substituents (by exposure to alkali) allows the polymer chains to associate and form a gel network (Maekaji, 1974, 1978). This gel has long been used in Japanese traditional foods (Nishinari, 1988). Although KGM is not a permitted food additive in the West, it has long history of food industrial use in Japan, which might facilitate approval by the United States Food&Drug Administration and the European Community (E.R.Morris., 1990b). KGM has also attracted much attention from a point of nutritional effects, because it could play a role as a dietary fiber.

KGM is a β-D-1,4 linked glucomannan and contains 1-3 linked branches occurring at the C3 position of glucose and mannose residues (Nishinari et al. 1992b), however, the physicochemical properties of KGM have not been clarified sufficiently, because KGM is insoluble in water so that it is difficult to obtain well-fractionated konjac glucomannan samples (Nishinari et al., 1987; Mitsuyuki et al., 1987). Recently the interaction of KGM with other hydrocolloids such as xanthan, and κ-carrageenan has been studied (Dea, 1979; Cairns et al., 1986; Cairns et al., 1987; Brownsey et al., 1988; Williams et al., 1991 and 1993; Kohyama et al.; 1993; Annable, 1994a). KGM interacts with non-gelling concentrations of both agarose and κ-carrageenan to give gels similar in rheological properties and melting point to those obtained with galactomannan (Dea et al., 1987). By mixing with xanthan gum, KGM forms gels at unusually low concentration and with unusually high melting points compared to those using galactomannan. In Japan, mixed gels of carrageenan with konjac glucomannan, are called 'konnyaku jelly', and used as low-calorie dessert jellies, much liked for their special texture.

KGM is virtually a neutral polysaccharide as it has few acetyl groups. Thus, gelation of KGM is not affected so much by ions as by molecular weight of the KGM. Kohyama et al.(1993) carried out rheological and DSC studies on the gelation of KGM/carrageenan mixtures using three konjac glucomannan fractions with different molecular weights. The elastic modulus at small deformation, the breaking stress and breaking strain of the mixed gels increased with increasing molecular weight of konjac glucomannan, however, the gel-sol transition temperatures were not influenced so much by molecular weight of konjac glucomannan. From these results, another new model was proposed : the main network of the mixture is formed by carrageenan molecules, and konjac glucomannan molecules interact with carrageenan molecules and form weak junction zones which contribute to the elasticity but not so much to thermal stability (Figure IV-1-4).

Since gellan gum (GELL) is a helix-forming polysaccharide, analogous to carrageenans (the detailed description of gellan gum has already been shown in session 1 of this Chapter I), it seemed likely that synergism might be observed in GELL/KGM mixtures, similar to that in carrageenan/KGM mixtures. However, there is less information available about GELL and KGM mixtures. Five fractions of KGM with different molecular weights, prepared by enzymatic degradation (ND,$M_w=1.17\times10^6$; LM-1,$M_w=9.5\times10^5$; LM-

1,Mw=1.17×10^5; LM-2,Mw=2.51×10^5; LM-3,Mw=8.1×10^4; LM-4,Mw=4.0×10^4), were kindly supplied by Shimizu Chemical Co.. In Chapter IV, the rheological and thermal properties of GELL/KGM mixtures are described using dynamic viscoelastic measurement and differential scanning calorimetry. The effects of molecular weight of the KGM on these properties are discussed. In Chapter V, the effects of monovalent and divalent cations on the interaction between GELL and KGM are described in order to elucidate the mechanism of interaction.

single polymer network containing the second polymer within the gel

interpenetrating networks

phase-separated networks

coupled networks

Fig.IV-1-1 Various types of network structures for binary polysaccharide gels.
a) a single polymer network with the second polymer entrapped
b) interpenetrating networks c) phase separated networks and
d) coupled networks. from Cairns et al. (1987)

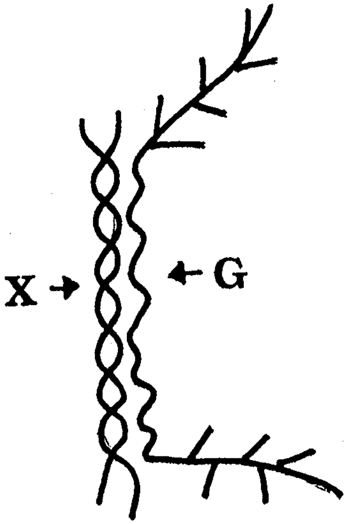

Fig. IV-1-2 Schematic representations of the molecular origin of xanthan interaction with galactomannan according to Dea et al. (1977)

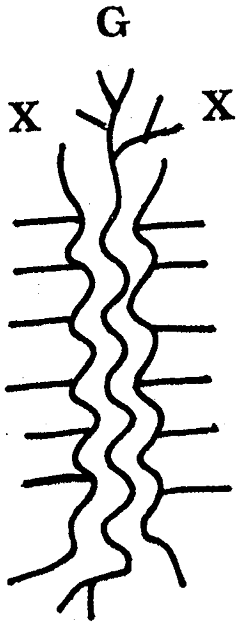

Fig. IV-1-3 Schematic representation showing the xanthan-galactomannan sandwich structure. From Cairns et al.(1987)

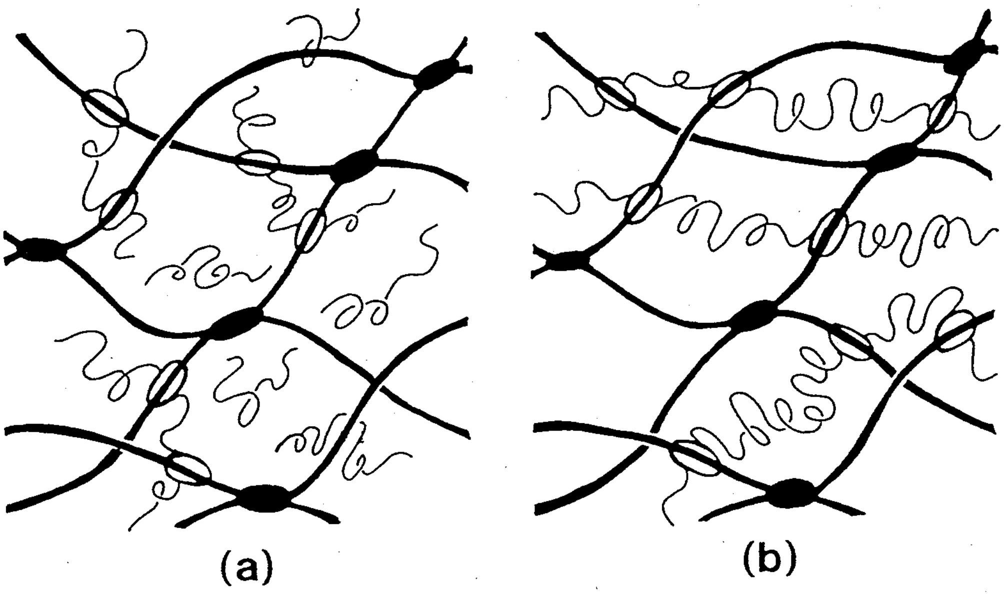

Fig. IV-1-4 The effect of molecular weight of konjac glucomannan (KGM) on the gel network structure. Mixed gels of carrageenan (CAR) and low molecular weight KGM(a), and high molecular weight KGM(b) Thick lines represent CAR molecular chains whilst thin lines show KGM chains. The number of elastically active chains and the contour length of active chains increase with increasing molecular weight of KGM. ●, junction zones made by CAR-CAR; ○, junction zones made by CAR-KGM. (Kohyama et al., 1993)

IV-2 Interaction between Gellan gum and Konjac Glucomannan

MATERIALS AND METHODS

Gellan gum used in this chapter was the same as described in Chapter I.

The non-degraded native konjac glucomannan (ND) and two fractions of konjac glucomannan with different molecular weights (LM-1 and LM-2), prepared by enzymatic degradation, were kindly supplied by Shimizu Chemical Co., Hiroshima, Japan. The molecular weights of the sample fractions determined by the same method in the previous work (Kohyama et al., 1993) were 1.17×10^6(ND), 9.5×10^5(LM-1) and 2.51×10^5(LM-2). Man/Glc ratio estimated from the peak area detected by refractive index did not depend so much on the molecular weight of KGM, and was about 2.0.

Solutions of gellan gum/konjac glucomannan at various ratios were prepared by mixing the appropriate amounts of each powder and dispersed by stirring to swell at 40℃ overnight. The total polysaccharide concentration was fixed at 1.6% (w/w) and the gellan gum/konjac glucomannan ratio was changed. Methods of rheological and DSC measurements were described in section 2 of Chapter I.

RESULTS

Rheological properties

Figure IV-2-1 shows the frequency dependence of G' and G" for a 1.6% solution of gellan gum alone at various temperatures. The viscoelastic behaviour was typical of a dilute polymer solution.

Figure IV-2-2 shows the frequency dependence of G' and G" for mixtures of gellan gum (GELL) and ND (total polysaccharide concentration 1.6%) with various mixing ratios and at various temperatures. For a mixture with GELL/ND=1.4/0.2 [Figure IV-2-2 (a)], the behaviour at 0℃ was typical of a concentrated polymer solution, i.e. G' was smaller at lower frequencies but became larger than G" at higher frequencies. At 15, 25, and 30℃, a typical dilute polymer solution behaviour was observed where G" is greater than G' at all frequencies. Mixtures with GELL/ND=1.0/0.6 [Figure IV-2-2(b)], 0.6/1.0 [Figure IV-2-2(c)], and a 1.6% solution of ND alone [Figure IV-2-2(d)] showed similar tendencies but the cross-over frequency observed at 0℃ shifted to lower frequencies with increasing content of ND. G' and G" of the 1.6% solution of ND alone is least frequency dependent and least temperature dependent. The temperature dependence was most prominent at lowest frequencies, and the difference between G' and G" at 0 ℃ and 30℃ increased with increasing content of gellan gum, indicating that this is induced by the conformational change of gellan molecules and that the conformation of ND does not change over this temperature range. This will be discussed later in more detail based on the thermal scanning rheological measurements.

Figure IV-2-3 shows the frequency dependence of G' and G" for mixtures of gellan gum (GELL) and LM-1 (total polysaccharide concentration 1.6%) with various mixing ratios and at various temperatures. For a mixture with GELL/LM-1=1.4/0.2 [Figure IV-2-3(a)], the behaviour at 30℃ or 25℃ was typical of a dilute polymer solution, however, the behaviour at 15℃ was typical of a concentrated polymer solution because G' approached G" at higher frequencies and in fact, G' and G" actually showed a cross-over at a relatively high frequency. The behaviour at 0℃ was also typical of a concentrated polymer solution and the cross-over frequency was lower than that at 15℃. The behaviour of a mixture with GELL/LM-1=1.0/0.6 [Figure IV-2-3(b)] showed a similar tendency to that of a mixture with GELL/LM-1=1.4/0.2 [Figure IV-2-3(a)]. For a mixture with GELL/LM-1=0.6/1.0 (Figure IV-2-3(c)), the behaviour even at 30℃ or 25℃ was typical of a concentrated polymer solution, moreover, the behaviour at 15℃ or 0℃ tended towards that of a weak gel. For a 1.6% LM-1 solution [Figure IV-2-3(d)], at any temperature from 30℃ to 0℃, the behaviour was typical of a concentrated polymer solution and those curves were less dependent on temperature.

Figure IV-2-4 shows the frequency dependence of G' and G" for mixtures of GELL/LM-2 at various mixing ratios and at various temperatures. G" is greater than G' at all frequencies and at all temperatures examined for mixtures with GELL/LM-2=1.4/0.2 [Figure IV-2-4(a)], 1.0/0.6 [Figure IV-2-4(b)] and G' was too small to be detected at all temperatures for a 1.6% solution of LM-2 alone [Figure IV-2-4(d)].

Figure IV-2-5 shows the storage modulus G' for GELL/ND mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0 rad/s and at various temperatures. At temperatures from 30℃ to 0℃, G' for mixtures increased monotonically with increasing content of ND in the mixtures, though the modulus for a mixture with GELL/ND=0.8/0.8 showed a shallow dip. G' for a 1.6% solution of ND alone was significantly larger than that for any other mixtures and was little dependent on temperature.

Figure IV-2-6 shows the storage modulus G' for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0rad/s and at various temperatures. The behaviour of G' as a function of LM-1 content in gellan gum/LM-1 mixtures depended on temperature. G' at 30℃ increased monotonically with increasing content of LM-1 in mixtures whilst G' at 25℃ was maximum at a mixing ratio of GELL/LM-1=0.4/1.2 and G' at 15℃ or 0℃ was maximum at GELL/LM-1=0.6/1.0.

Figure IV-2-7 shows the storage modulus G' for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0rad/s and at various temperatures. The behaviour in GELL/LM-2 mixtures was significantly different from that in GELL/LM-1 or GELL/ND mixtures with the same total polymer concentration. G' as a function of LM-2 content at 0℃ slightly increased up to LM-2 content 0.4 and then rapidly decreased with increasing content of LM-2 in the mixtures. At 15℃, 25℃ and 30℃, G' became maximum at LM-2 content 0.2~0.4. G' at any temperature for mixtures with LM-2 content more than 1.4 was too small to be detected. Thus, in the case of GELL/LM-2 mixtures, a significant decrease in G' with increasing concentration of LM-2 occurred, so that it seems that G' for the GELL/LM-2 mixtures is dominated by the modulus of the gellan gum. It appeared that synergism occurred with LM-2 only at lower konjac glucomannan content around 0.2.

Figure IV-2-8 shows the storage modulus G' for GELL/ND, GELL/LM-1 and GELL/LM-2 (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0rad/s and at 30℃ [Figure IV-2-8(a)] and at 0℃ [Figure IV-2-8(b)]. Closed diamond represents G' for gellan alone. At all the mixing ratios and at both temperatures, G' for gellan alone is smaller than that for mixtures. The crosses represent G' for KGM alone, +:ND and ┼:LM-1. At the mixing ratios with higher KGM contents, values of G' for mixtures were larger than those for KGM alone. The synergistic interaction seems to be important at lower temperatures.

In order to see whether molecular association between gellan and konjac glucomannan occurs or not, the following experiment was carried out; the storage shear modulus G' of GELL/KGM mixtures with constant GELL concentration 1% and various LM-2 concentrations was observed. Figure IV-2-9 shows that G' increased with increasing LM-2 concentration up to ~0.3% but then decreased, indicating the occurrence of phase separation due to thermodynamic incompatibility (Dea and Morrison, 1975; Watase and Nishinari, 1980; Clark et al, 1982, Clark et al. 1983; Clark and Ross-Murphy, 1985; Clark and Lee-Tuffnell, 1986, Leloup et al., 1990; Annable et al., 1994b).

Figure IV-2-10 shows the temperature dependence of G' and G'' during a cooling or a heating process for a 1.6% solution of gellan gum alone at 0.1rad/s at a cooling or heating rate of 0.5℃/min. During the cooling process, G' for a 1.6% solution of gellan gum alone could be detected below around 26℃, corresponding to the setting temperature T_s, and increased gradually with decreasing temperature, while G'' could be detected from 50 ℃ and increased rapidly at~35℃ and then more gradually. During the heating process, G' for a 1.6% solution of gellan gum alone decreased gradually with increasing temperature and then became too small to be detected, corresponding to the melting temperature T_m, although G'' began to decrease rapidly at ~30℃ and then more gradually.

Figure IV-2-11 shows the temperature dependence of G' and G'' during the cooling or heating process for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios and at 0.1 rad/s and at 0.5℃ /min. The thermal behaviour of G' and G'' for a mixture with GELL/ND=1.4/0.2 [Figure IV-2-11(a)] was similar to that for a gellan gum alone (Figure IV-2-10), although the temperature at which G' becomes detectable in the cooling process shifted to higher temperatures. Thermal hysteresis was small for a mixture with GELL/ND=1.0/0.6 [Figure IV-2-11(b)] or 0.6/1.0 [Figure IV-2-11(c)], and G' and G'' became less temperature dependent with increasing content of ND. For a 1.6% solution of ND alone [Figure IV-2-11(d)], both moduli were significantly larger throughout the accessible temperature range than those of any other mixtures [Figure IV-2-11(a)~(c)] and became less temperature dependent.

Figure IV-2-12 shows the temperature dependence of G' and G'' during the cooling or heating process for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at 0.1rad/s and at a cooling or heating rate of 0.5℃/min. The thermal behaviour of G' and G'' for a mixture with GELL/LM-1=1.4/0.2 [Figure IV-2-12(a)] was similar to that for a gellan gum alone. However, the temperature at which G' begins to be detected in the cooling process shifted to higher temperatures and G' and G'' increased with increasing content of LM-1. G' for a mixture with GELL/LM-1=1.0/0.6 [Figure IV-2-12(b)] could be also detected even at 50℃ in both cooling and heating process, and G' and G'' became less temperature dependent. Moreover, the curves of both moduli in the cooling process almost coincided with those in the heating process, so that the thermal hysteresis was little exhibited. G' and G'' for a 1.6% solution of LM-1 alone [Figure IV-2-12(d)] were significantly larger throughout the accessible temperature range than those of any other mixtures [Figure IV-2-12(a)~(c)] and became less temperature dependent.

Figure IV-2-13 shows the temperature dependence of G' and G'' during the cooling or heating process for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. The thermal behaviour of G' and G'' for GELL/LM-2 mixtures was significantly different from that for GELL/ND mixtures (Figure IV-2-11) or GELL/LM-1 mixtures (Figure IV-2-12). Mixtures with GELL/LM-2=1.4/0.2 [Figure IV-2-13(a)] and 1.0/0.6 [Figure IV-2-13(b)] exhibited thermal hysteresis, however, the temperature at which G' begins to be detected in the cooling process and the temperature at which G' becomes too small to be detected in the heating process shifted to lower temperatures with increasing content of LM-2. Moreover, the values of G' and G'' for these mixtures GELL/LM-2 became smaller with increasing content of LM-2. G' for a mixture with LM-2 content 1.0% [Figure IV-2-13(c)] was too small to be detected, and the temperature at which G'' increased steeply shifted to a lower temperature and G'' became less temperature dependent with increasing content of LM-2.

DSC

Figure IV-2-14 shows cooling and heating DSC curves for gellan gum/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios of GELL/LM-1. The cooling curve for a 1.6% solution of gellan gum alone [Figure IV-2-14(a)] showed a main exothermic peak at 32.3℃ and another small peak at 95.1℃ whilst the heating curve [Figure IV-2-14(b)] showed only a main endothermic peak at 33.7℃. The cooling or heating curves for all mixed GELL/LM-1 solutions also showed a main exothermic or a main endothermic peak at a temperature range from 15 to 35℃, however, both the exothermic peak temperature T_s and the endothermic peak temperature T_m shifted to lower temperatures with increasing content of LM-1 in mixtures. The midpoint temperature of transition T_M determined from rheological measurements seems to be inbetween T_s and T_m determined by DSC measurements. The exothermic and endothermic enthalpies for mixtures decreased with increasing content of LM-1 in mixtures, moreover, for mixtures where LM-1 is in excess, both exothermic peak and endothermic peak became very broad. Eventually, the cooling or heating DSC curve for a 1.6% solution of konjac mannan (LM-1) alone [Figure IV-2-14(a) and (b)] did not show any exothermic or endothermic peak, indicating that konjac glucomannan alone does not gel or does not show any conformational change such as helix-coil transition, and is consistent with rheological results mentioned above, which agreed with previous results reported by many other workers. In the case of ND or LM-2, cooling and heating DSC curves for mixtures showed a similar tendency to those of gellan gum/LM-1 mixtures (data not shown), i.e. the exothermic peak temperature T_s in cooling DSC curves as well as the endothermic peak temperature T_m in heating DSC curves shifted to lower temperatures with increasing content of KGM.

The experimental findings that both T_s and T_m for the mixtures with 1.6% total polysaccharide concentration shifted to lower temperatures with decreasing gellan gum content may be ascribed to the decrease of gellan content itself and/or to the inhibition of helix formation of gellan by konjac glucomannan.

Figure IV-2-15 shows the dependence of T_s(a) or T_m(b) for the gellan gum/various konjac mannan mixtures as a function of mixing ratio, compared to those for solutions of gellan gum alone at various concentrations. It is clearly demonstrated that the T_s or T_m of gellan gum/konjac glucomannan mixed systems in the DSC measurement was shifted to lower temperatures with increasing content of konjac glucomannan whatever its molecular weight. This may suggest that the ordered structure was little dependent on the molecular weight of konjac glucomannan. However, as stated above, it was shown that the temperature and frequency dependence of moduli for gellan gum/konjac glucomannan mixtures showed a strong dependence on the presence and the molecular weight of konjac glucomannan. Therefore, the effects of molecular weight of konjac glucomannan would be more significant in rheological properties than in thermal properties as in the case of κ-carrageenan/konjac glucomannan mixed systems reported previously (Kohyama et al., 1993). In all the cases, at lower concentrations of gellan gum (hence higher content of konjac mannan), both T_s and T_m for mixed systems were slightly higher than those for gellan gum itself. When the concentration of gellan gum in the mixture reached 0.8% (the mixing ratio of GELL/KGM reached 1:1), both T_s and T_m for the mixed system were slightly lower than those for gellan gum alone. At higher concentrations of gellan gum (hence lower content of konjac mannan), both T_s and T_m for the mixed systems were slightly lower than those for gellan gum alone although both T_s and T_m for the mixture with GELL/LM-1=1.4/0.2 were slightly higher than those for gellan gum alone. Therefore, the shift of T_s and T_m to lower temperatures for the mixtures in which KGM content is higher than 50% is attributed to the decrease of gellan content and it is noteworthy that the excessive KGM promotes the coil-helix transition of gellan. It is possible to expect some synergistic interaction at higher KGM content as also shown in the rheological results (Figure IV-2-6). On the other hand, the shift of T_s and T_m to lower temperatures for the mixtures in which KGM content is lower than 50% is attributed to the inhibition of gellan helix formation by KGM. In all the cases, T_s and T_m for the mixed systems decreased in the order of ND, LM-1, LM-2.

DISCUSSION

The difference in behaviours of 1.6% solutions of KGM with different molecular weights [Figure IV-2-2(d), Figure IV-2-3(d), Figure IV-2-4(d)] is induced by a difference in the entanglement which is often observed for flexible macromolecules such as poly(methyl methacrylate) (Masuda et al. 1970) and polystyrene (Onogi et al. 1970). The cross-over frequency shifted to lower frequencies with increasing molecular weight of KGM [Figure IV-2-2(d) and Figure IV-2-3(d)] as in poly(methyl methacrylate) or polystyrene molecules, although these macromolecules are slightly more flexible than KGM.

Although the highest molecular weight fraction ND seems to inhibit the helix formation of gellan more, the storage modulus of GELL/ND mixtures as a function of ND content (Figure IV-2-5) increases with increasing ND content. This is simply because G' of a solution of ND alone is far larger (more than two decades!) than that of gellan alone. The inhibition seems to be most important at a mixing ratio of GELL/ND=0.8/0.8. Since ND inhibits most the helix formation of gellan, no synergistic interaction was observed in these mixtures (Figure IV-2-5).

The findings that G' of GELL/LM-1 mixtures as a function of mixing ratio showed a maximum only below 25℃ as shown in Figure IV-2-6 suggest that the synergistic interaction occurs only between gellan gum molecules in helix conformation and konjac glucomannan because gellan gum molecules take a helical conformation at lower temperatures.

The reason for the increase of G' by addition of KGM may be ascribed to one of the following three possibilites: 1) An increase of effective concentration of gellan due to volume exclusion. 2) Segregation due to thermodynamic incompatibility. 3) Association of gellan and KGM. The synergistic interaction occurs only at lower temperatures around GELL/LM-1 ratio 0.6/1.0~0.4/1.2, and at wider temperature range for GELL/LM-2 around the mixing ratio 1.4/0.2~1.2/0.4 as shown in Figures IV-2-6 and IV-2-7. The reason why GELL/LM-2 mixtures show the synergistic interaction over a wider temperature range as well as a wider mixing ratio may be attributed to the least inhibition of helix formation of gellan molecules by KGM of the lowest molecular weight. If molecular association occurs between gellan and KGM, the increase in G' should be more prominent in a mixture with higher molecular weight KGM, however, this was not the case. Since the synergism between gellan gum and konjac glucomannan does not seem to contribute so much to the increase of G', the first or the second possibility is more plausible.

As was described above, the behaviours of the mixtures of 1.6% total polysaccharide concentration are characteristic of dilute or concentrated polymer solutions. Therefore, the transition observed from the temperature dependence of G' and G'' should not be attributed to a sol-gel transition. This may be induced by the coil-helix transition. The midpoint temperature T_M of the transition shifted to lower temperatures and the relaxational strength ΔG at T_M decreased monotonically with increasing content of KGM.

As is seen from Figures IV-2-11~IV-2-13, the temperature dependence of moduli for GELL/KGM mixtures is remarkably dependent on molecular weight. In all three cases, the midpoint transition temperature T_M shifted to lower temperatures, and the relaxation strength ΔG decreased with increasing content of KGM [Figure IV-2-16(a) and (b)]. T_M and ΔG for gellan alone is also shown in this Figure IV-2-16 (a) and (b) for the comparison with those for mixtures. At all the mixing ratios and for all the KGM fractions, ΔG for gellan alone is larger than that for mixtures. This is in good agreement with DSC results shown in Figure IV-2-15. Since the main contribution to the magnitude of ΔG is by the formation and the subsequent aggregation of helices of gellan molecules, this suggests that the process is inhibited by the presence of KGM. Another possibility that the decrease in ΔG with increasing KGM content is caused by the decrease in gellan content should be excluded because ΔG for mixtures with the same gellan content is smaller than that for gellan alone of the same concentration. The relaxation strength ΔG decreased with increasing molecular weight of KGM, therefore, the inhibition becomes more important with increasing molecular weight of KGM. However, as is shown in Figures IV-2-6 and IV-2-7, some synergistic effects were observed at a certain mixing ratio where G' for a mixture is larger than G' for a pure component at lower temperatures. The fact that the endothermic peak temperature T_m in a heating DSC curve is higher than the exothermic peak temperature T_s in a cooling DSC curve as shown in Figure IV-2-15 is commonly observed for many thermoreversible systems. This is due to chain aggregation and can be understood by a zipper model approach (Nishinari et al., 1990). As reported previously, however, the extrapolation of scan rate to zero will make this difference between T_m and T_s negligible for gellan gum (Manning, 1992; Miyoshi et al., 1994b). Both these exothermic and endothermic peaks should not be attributed to the gel-sol transition because in all these cases the frequency dependence described above shows that these mixed systems are not in a gel state but in sol state.

The fact that a cooling DSC curve shows two exothermic peaks whilst a heating DSC curve shows only one endothermic peak has been observed for a thermoreversible gel system (Williams et al., 1993). The higher temperature exothermic peak in the cooling DSC curves for GELL/LM-1 mixtures in Figure IV-2-13(a) may be attributed to the formation of ordered structure by the presence of small amount of calcium ions. As was shown in section 1 of Chapter II, an exothermic peak in cooling DSC curves splits into multiple peaks in the presence of excessive cations. It is improbable that helix-coil transition of gellan molecules shifted to such a higher temperature on addition of konjac glucomannan.

Figure IV-2-17 shows the exothermic enthalpy (a) and endothermic enthalpy (b) for GELL/KGM mixtures as a function of mixing ratio. The value of enthalpy is determined from the area enclosed by a DSC curve and a baseline, and divided by mass of gellan (kg) contained in the mixture. Both exothermic enthalpy and endothermic enthalpy per unit mass of gellan decreased with decreasing gellan concentration both for a system of gellan alone and for mixtures. The decrease in the enthalpy for gellan alone with decreasing gellan concentration indicates that the molecular association between gellan molecules is very strong with comparison to other gelling polysaccharides such as agarose in which the enthalpy per unit mass is independent of the concentration of gels (Watase and Nishinari et al., 1987). Although the data scatter a little, the enthalpy decreases by the addition of KGM at lower content of KGM and it showed the opposite tendency at higher content of KGM. This agrees fairly well with the tendency observed in the rheological measurements shown in Figure IV-2-16 and in Figures IV-2-6 and IV-2-7. In most cases, KGM inhibit the formation of ordered structure of gellan, but KGM promotes the structure formation at higher content of KGM. The inhibition of orderd structure is most conspicious on addition of the highest molecular weight KGM at lower KGM content, which is again consistent with rheological results.

Recently, it was suggested that the interaction between konjac glucomannan and κ-carrageenan is weaker than that between κ-carrageenan and κ-carrageenan, but strong enough to produce another elastically active chains (Kohyama et al., 1993). It is difficult to understand why gellan/konjac glucomannan does not show the similar synergism observed in κ-carrageenan/konjac glucomannan although gellan and κ-carrageenan show quite a similar gelling behaviour. The relation between the structure of polysaccharides and the synergistic interaction should be explored in the future. In conclusion, in GELL/KGM mixtures, the main ordered structure is formed by gellan molecules, and konjac glucomannan molecules inhibit gellan molecules to form an ordered structure. However, the present data were obtained only from rheological and thermal measurements, thus, more information from other methods are urgently necessary.

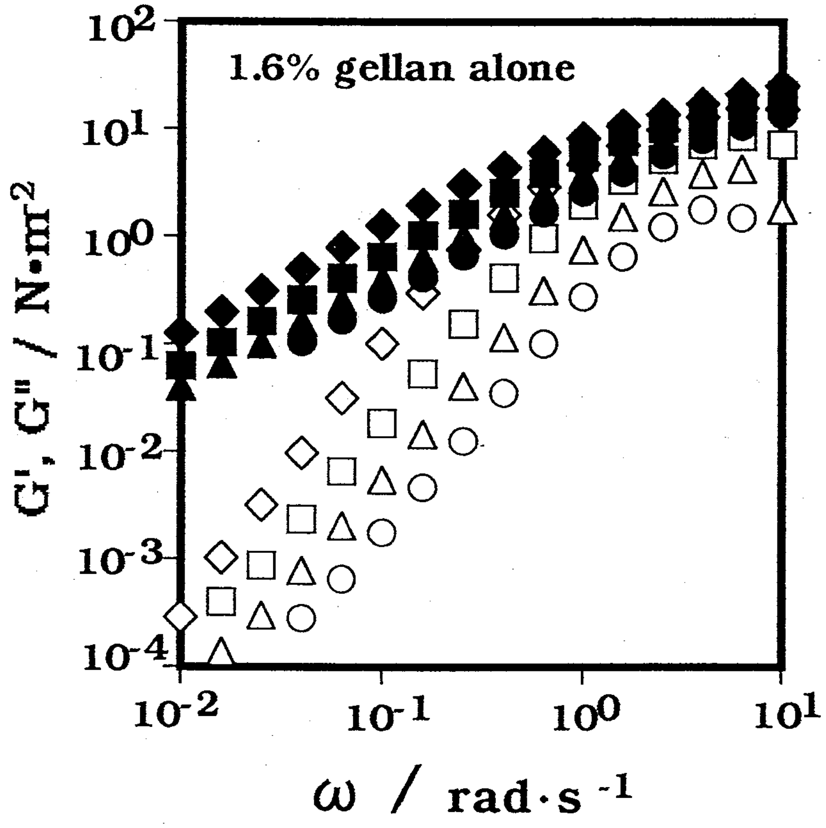

Fig. IV-2-1 Frequency dependence of storage modulus G' and loss modulus G" for a 1.6% solution of gellan gum alone at various temperatures. (○)G',(●)G",30℃; (△)G',(▲)G",25℃; (□)G',(■)G",15℃; (◇)G',(◆),G",0℃;

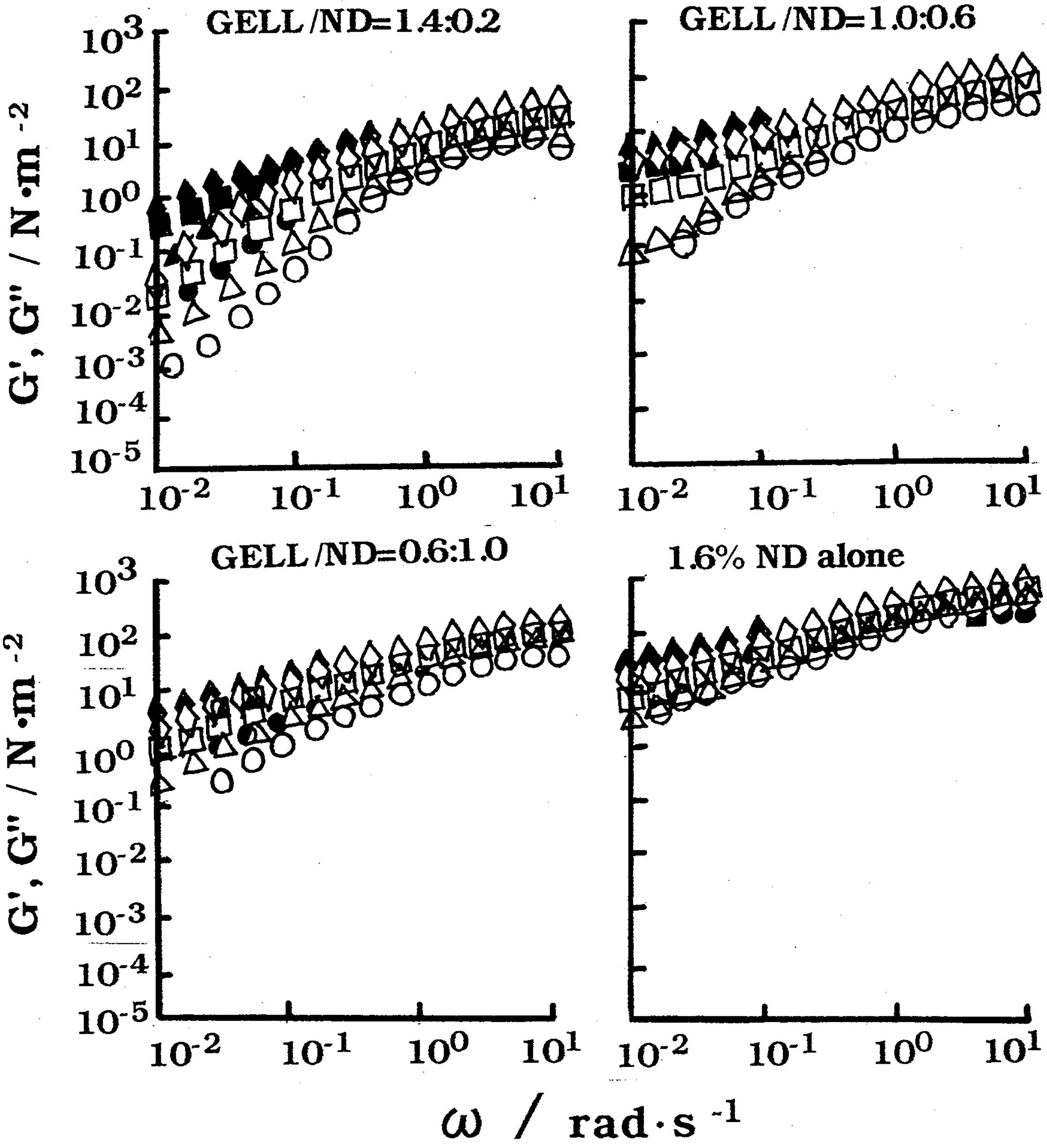

Fig. IV-2-2 Frequency dependence of storage modulus G' and loss modulus G" for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○)G', (●)G", 30℃; (△)G', (▲)G", 25℃; (□)G', (■)G", 15℃; (◇)G', (◆), G", 0℃; mixing ratios of GELL/ND:(A)1.4:0.2, (B)1.0:0.6, (C)0.6:1.0, (D)0.0:1.6.

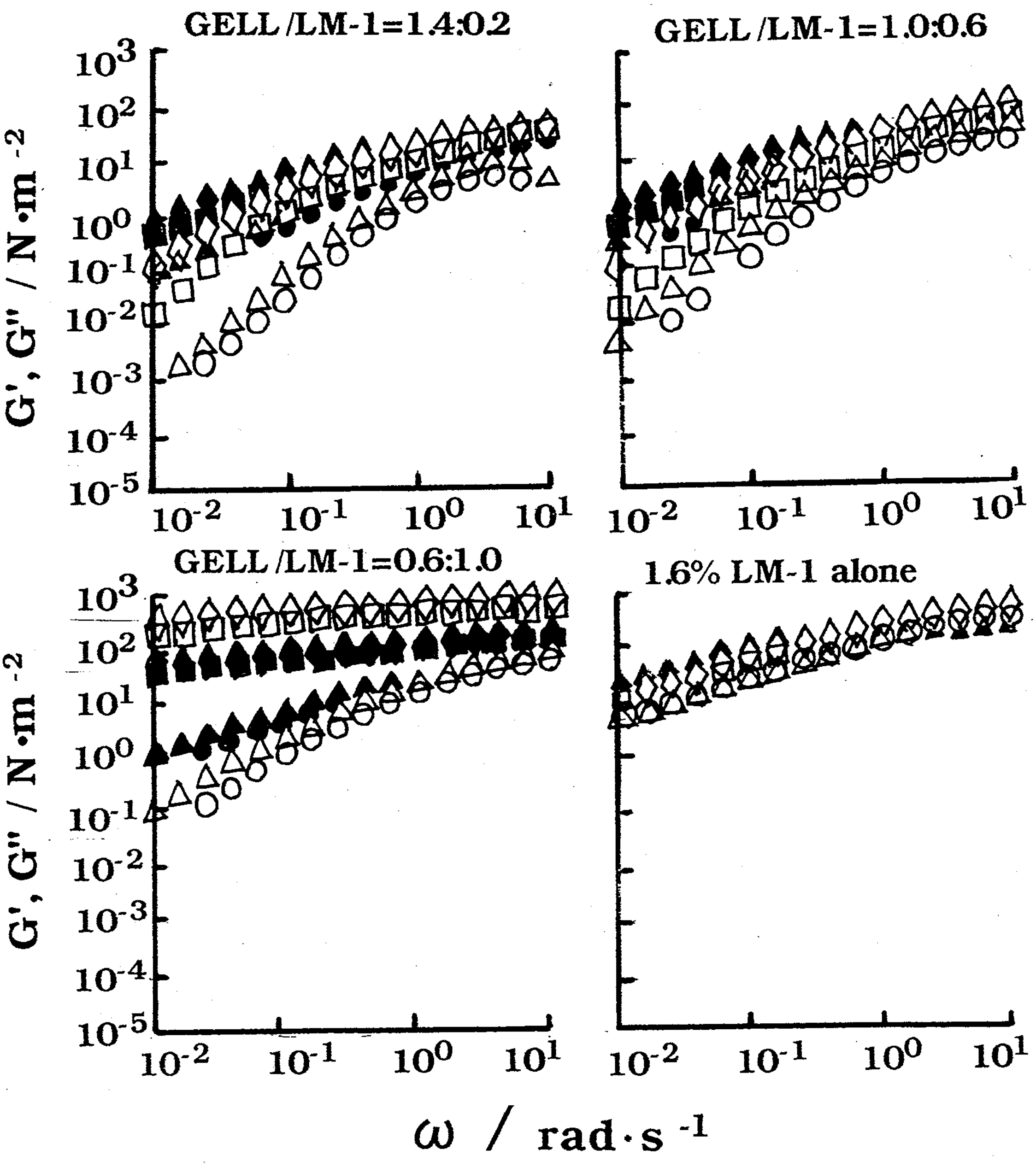

Fig. IV-2-3 Frequency dependence of storage modulus G' and loss modulus G" for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○)G',(●)G", 30℃; (△)G',(▲)G",25℃; (□)G',(■)G",15℃; (◇)G',(◆),G",0℃; mixing ratios of GELL/LM-1:(A)1.4:0.2, (B)1.0:0.6, (C)0.6:1.0, (D)0.0:1.6.

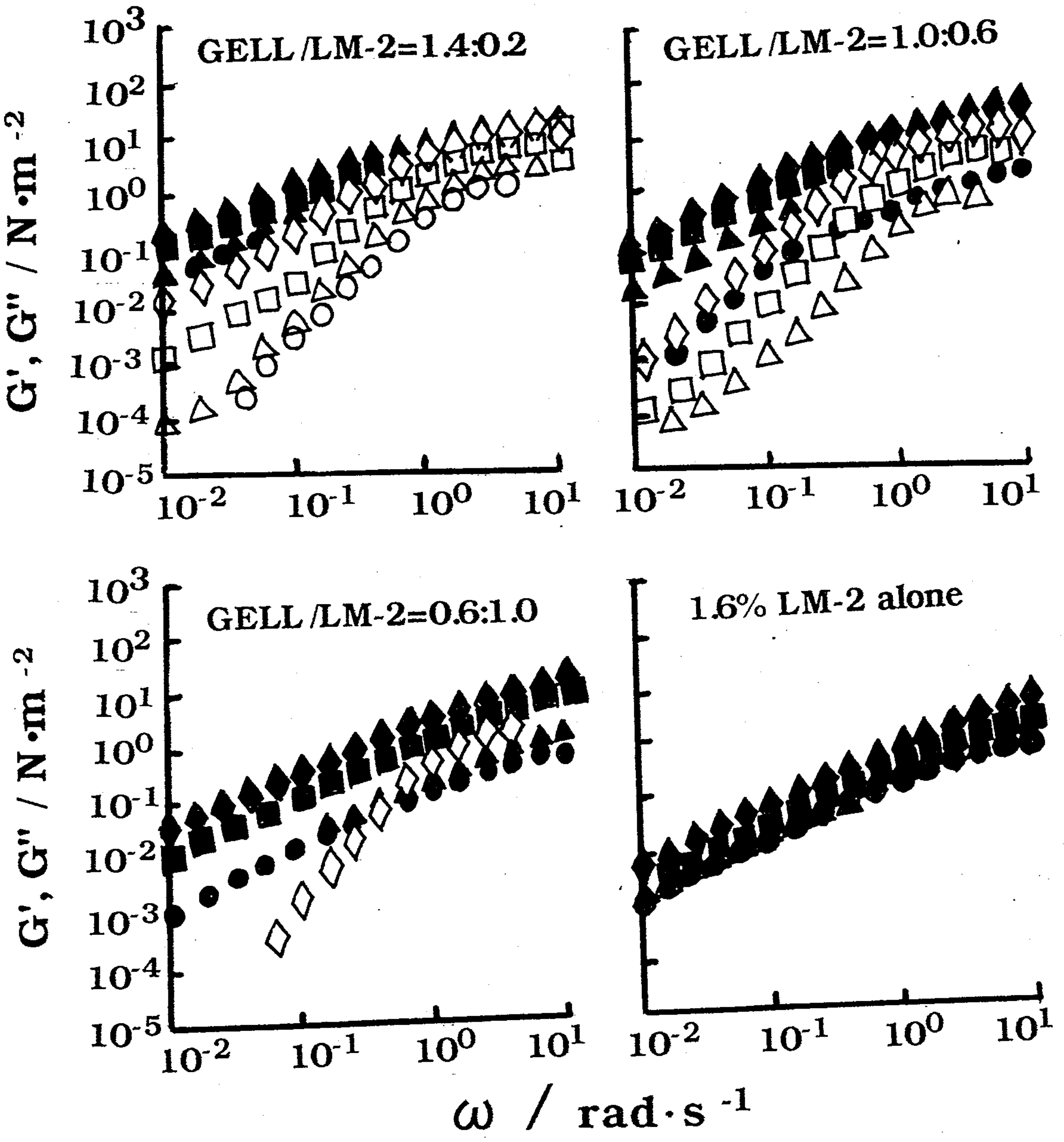

Fig. IV-2-4 Frequency dependence of storage modulus G' and loss modulus G" for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○)G',(●)G",30℃; (△)G',(▲)G",25℃; (□)G',(■)G',15℃; (◇)G',(◆),G",0℃; mixing ratios of GELL/LM-2:(A)1.4:0.2, (B)1.0:0.6, (C)0.6:1.0, (D)0.0:1.6.

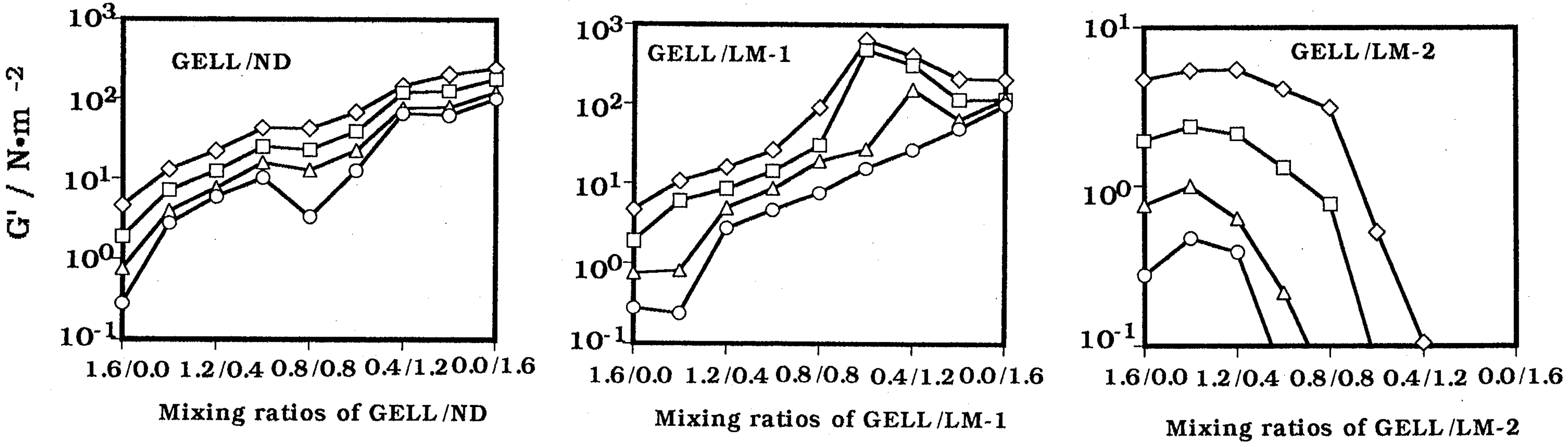

Fig.IV-2-5, 6, 7 Storage modulus G' for GELL/ND mixtures (left), GELL/LM-1 mixtures (center), GELL/LM-2 mixtures (right) (total polysaccharide concentration 1.6%) as a function of mixing ratio at various temperatures. (○)30℃, (△)25℃, (□)15℃, (◇)0℃; freq. $\omega = 1.0$ rad/s.

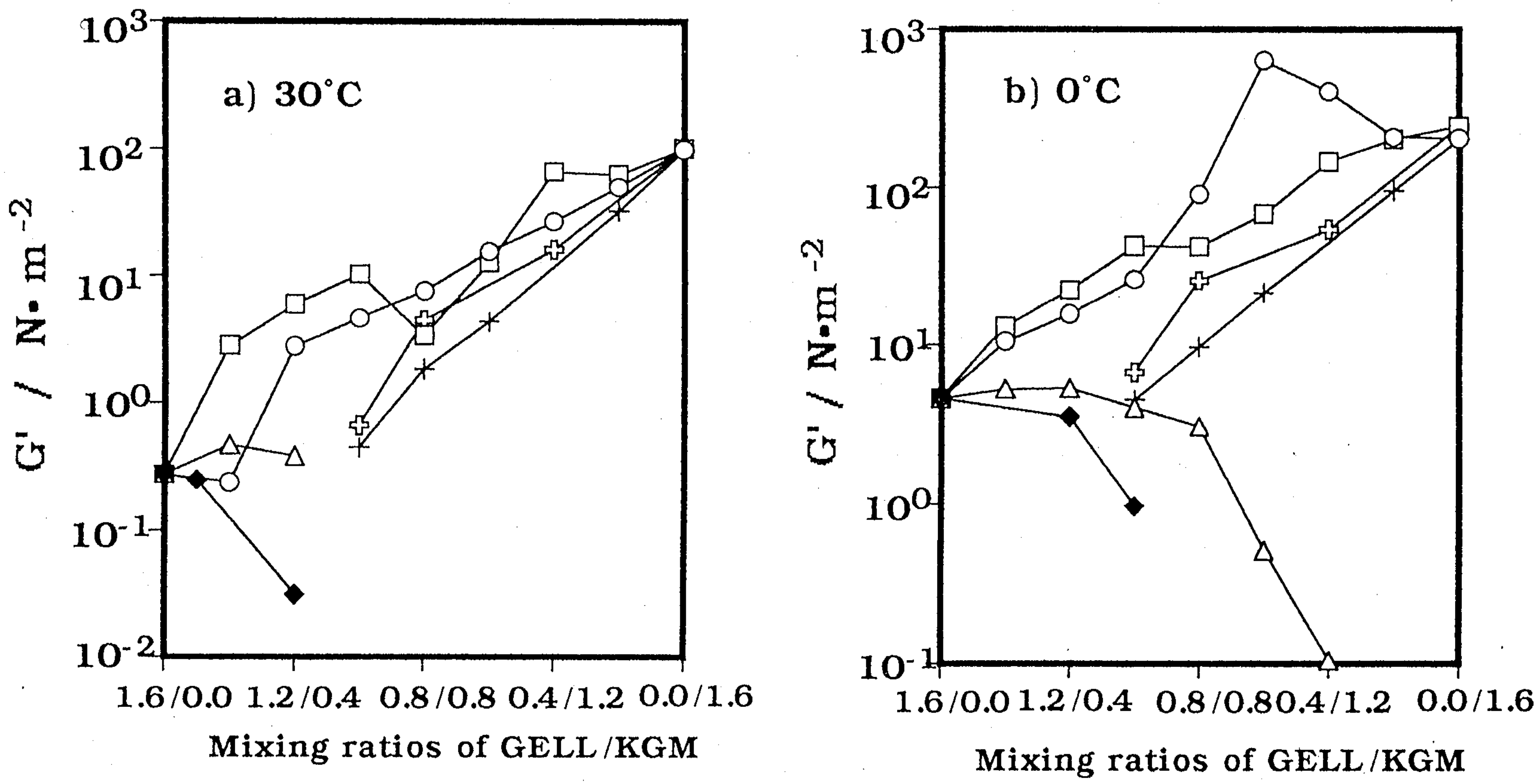

Fig. IV-2-8 Storage shear modulus G' of GELL/KGM mixtures (total polysaccharide concentration 1.6%), gellan gum alone and konjac glucomannan alone as a function of mixing ratio at 30℃(a) and at 0℃(b). (◆)gellan gum alone, (△)GELL/LM-2 mixtures, (○)GELL/LM-1 mixtures, (□)GELL/ND mixtures, (+)LM-1 alone, (✣)ND alone.

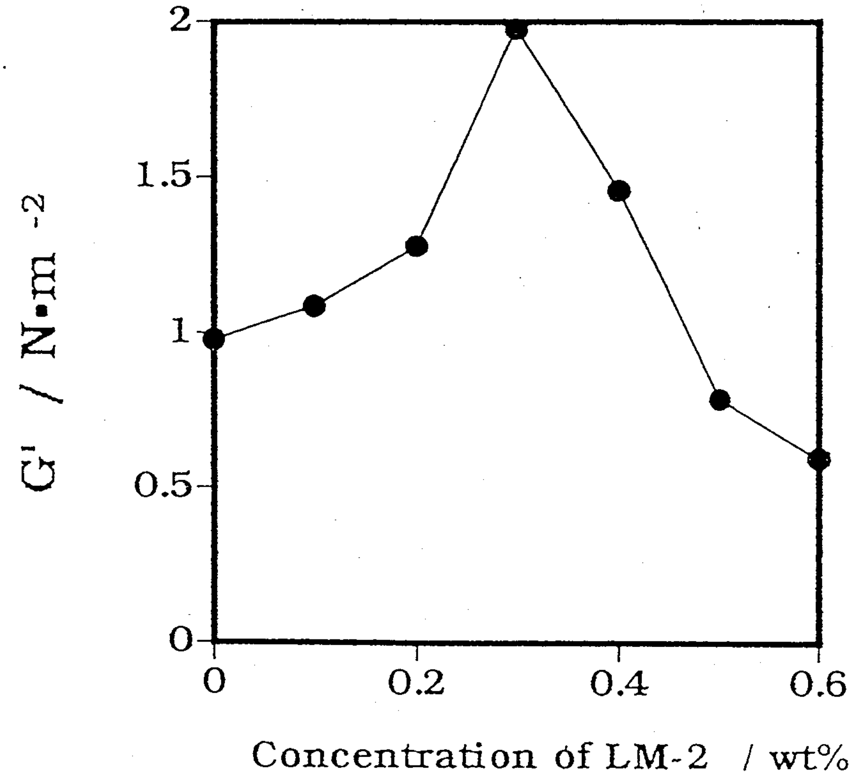

Fig. IV-2-9 Storage shear modulus G' of GELL/KGM mixtures with a constant concentration of GELL(1%) and various concentration of LM-2 as a function of LM-2 concentration at 0℃. freq.=1.0rad/sec.

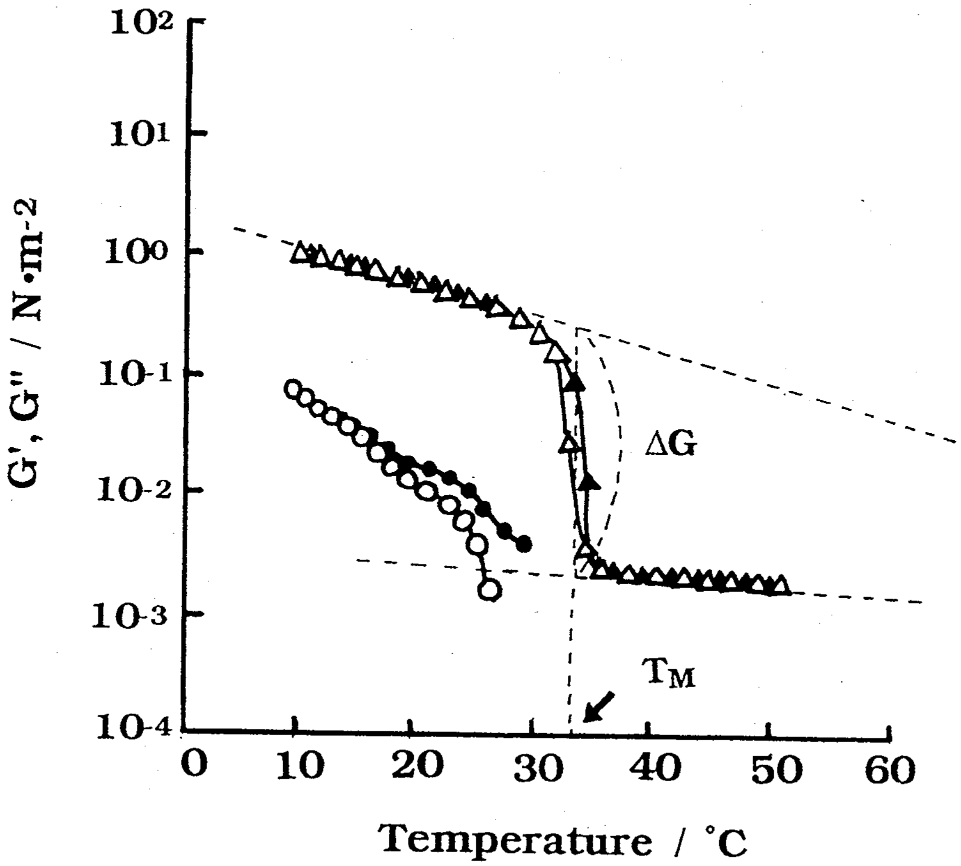

Fig. IV-2-10 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for a 1.6% solution of gellan gum alone. (○)G',(△)G", cooling; (●)G',(▲)G", heating; cooling and heating rate:0.5℃/min; freq.ω =0.1rad/s; ΔG represents the relaxational strength, and T_M stands for the midpoint temperature of the transition.

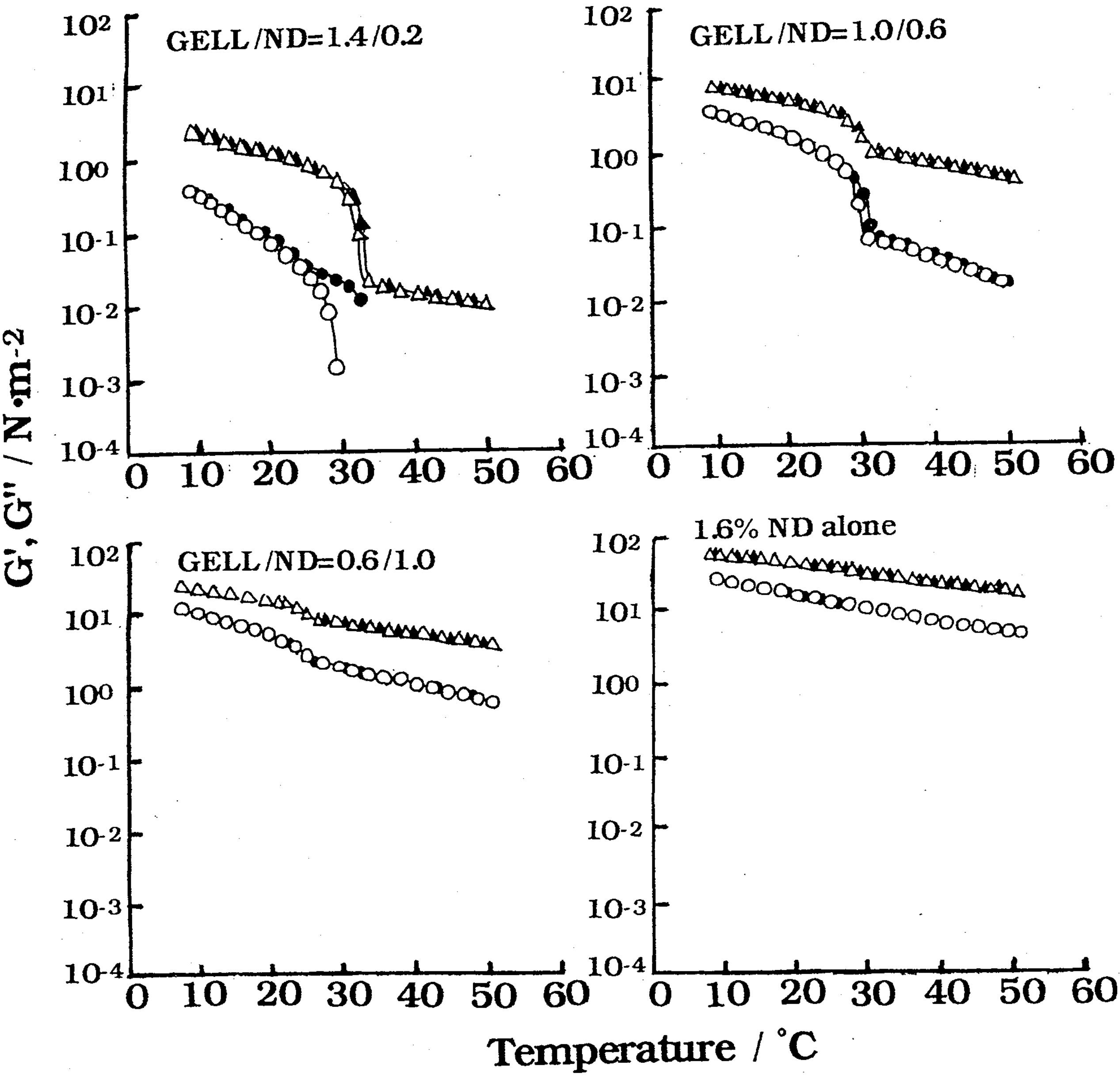

Fig. IV-2-11 Temperature dependence of storage modulus G' and loss modulus G'' during cooling or heating process for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○)G',(△)G'', cooling; (●)G',(▲)G'', heating; cooling and heating rate:0.5℃/min; freq.ω =0.1rad/s; mixing ratios of GELL/ND: (a)1.4:0.2, (b)1.0:0.6, (c)0.6:1.0, (d)0.0:1.6.

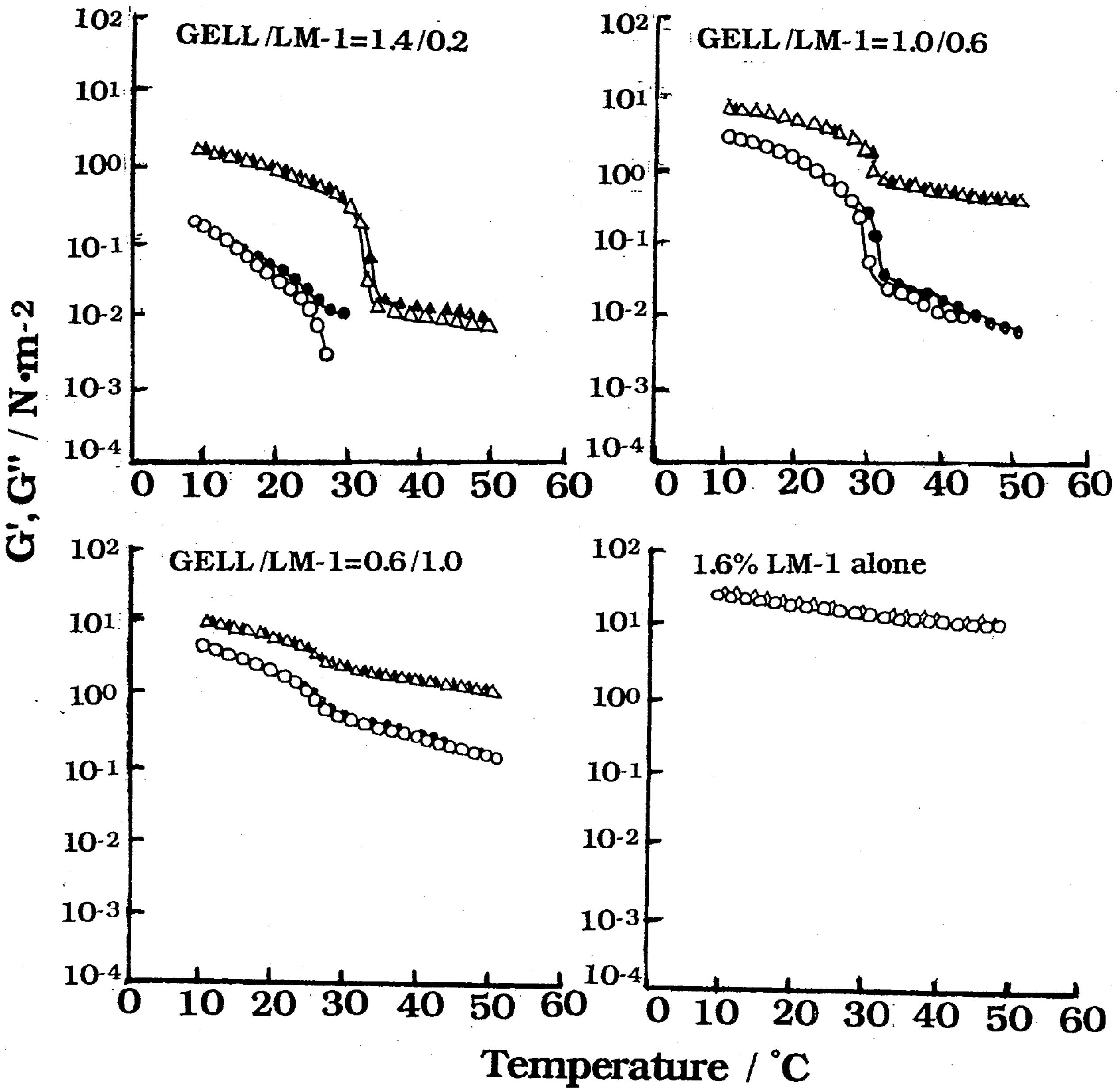

Fig. IV-2-12 Temperature dependence of storage modulus G' and loss modulus G" during cooling or heating process for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○)G',(△)G", cooling; (●)G',(▲)G', heating; cooling and heating rate:0.5℃/min; freq.ω=0.1rad/s; mixing ratios of GELL/LM-1: (a)1.4:0.2, (b)1.0:0.6, (c)0.6:1.0, (d)0.0:1.6.

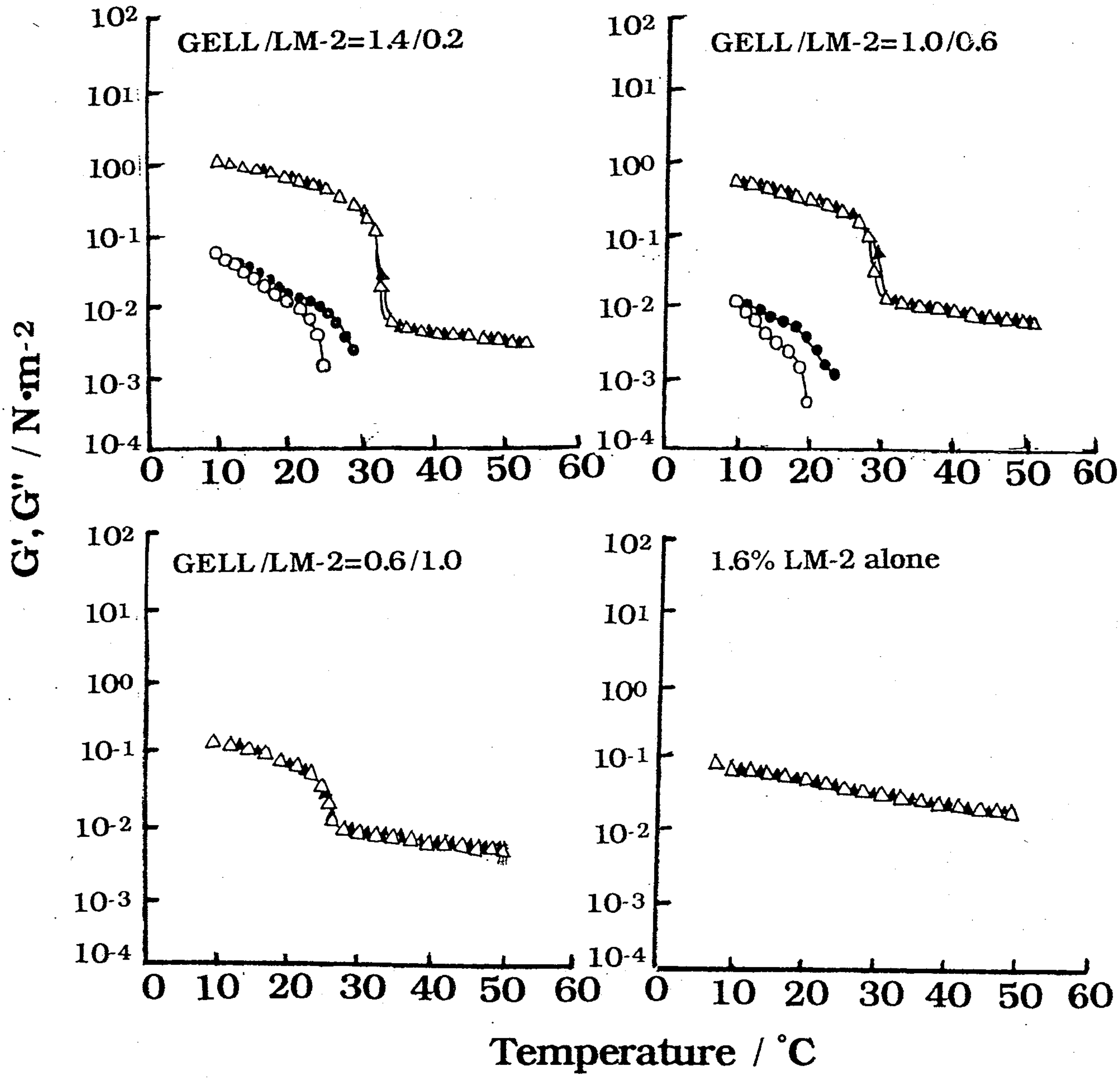

Fig. IV-2-13 Temperature dependence of storage modulus G' and loss modulus G'' during cooling or heating process for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○)G', (△)G'', cooling; (●)G', (▲)G'', heating; cooling and heating rate:0.5℃/min; freq.ω=0.1rad/s; mixing ratios of GELL/LM-2: (a)1.4:0.2, (b)1.0:0.6, (c)0.6:1.0, (d)0.0:1.6.

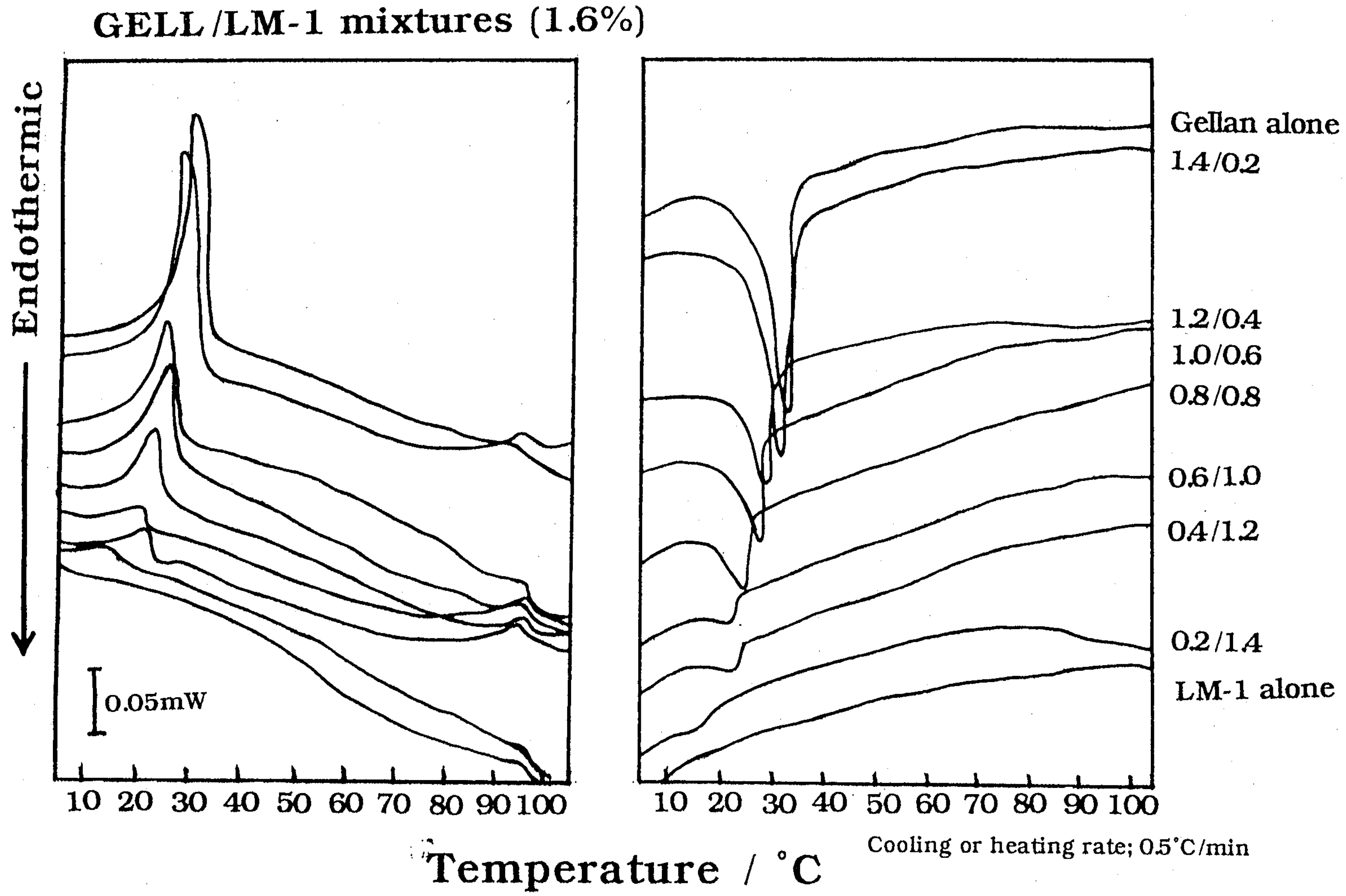

Fig. IV-2-14 Cooling (a) and heating (b) DSC curves for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. Cooling and heating rate: 0.5℃/min;

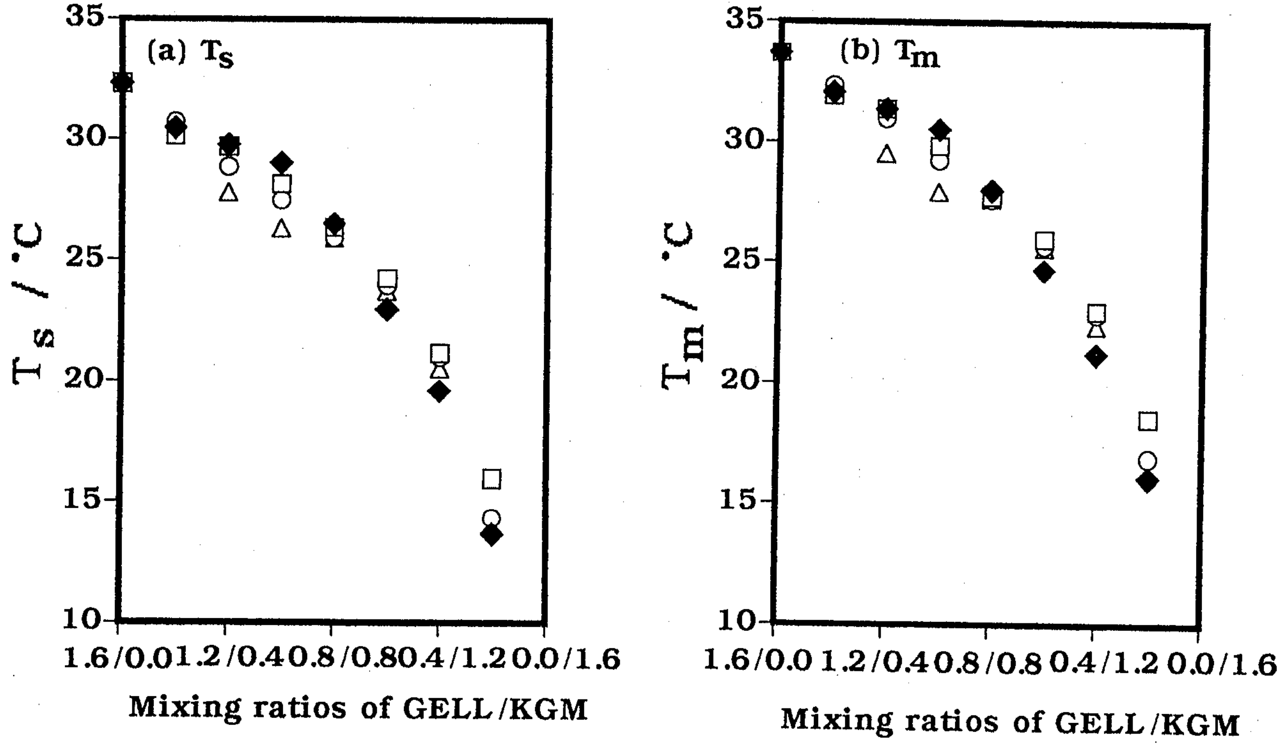

Fig. IV-2-15 Exothermic peak temperature T_s(a) and endothermic peak temperature. T_m(b) for a solutions of gellan gum alone and GELL/KGM mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio. (◆)gellan gum alone, (□)GELL/ND mixtures, (○)GELL/LM-1 mixtures, (△)gellan/LM-2 mixtures.

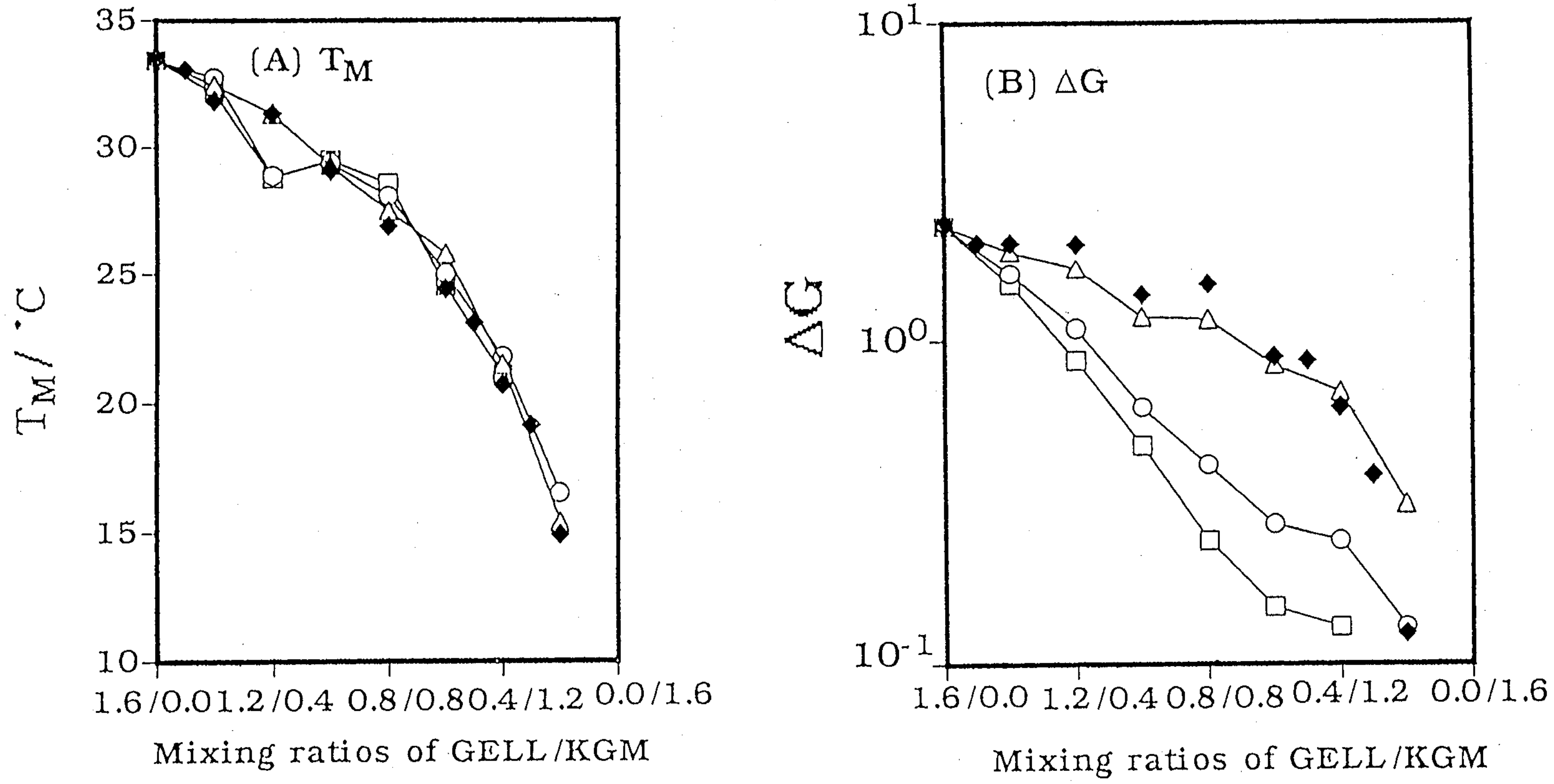

Fig. IV-2-16 Midpoint temperature T_M (a) and the relaxation strength ΔG (b) for GELL/KGM mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio. (◆)gellan gum alone, (□)GELL/ND mixtures, (○)GELL/LM-1 mixtures, (△)GELL/LM-2 mixtures.

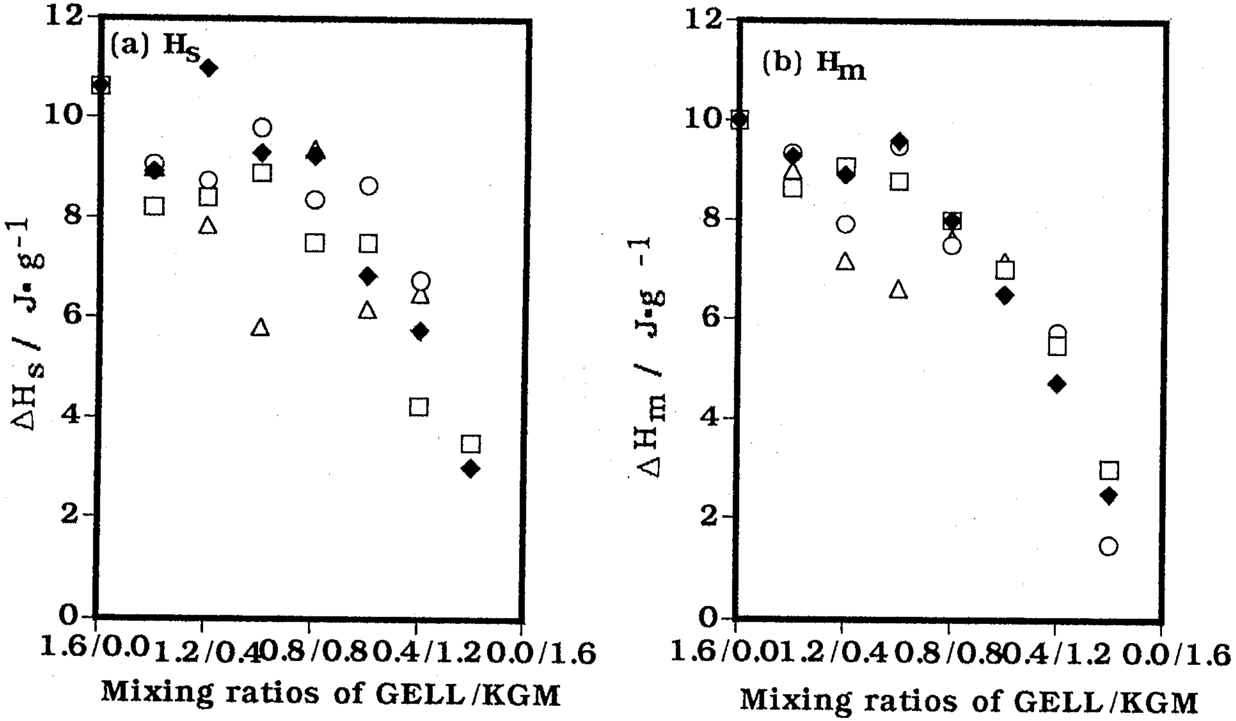

Fig. IV-2-17 Exothermic (a) and endothermic (b) enthalpy per unit mass of gellan for GELL/KGM mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio. ◆)gellan gum alone, (□)GELL/ND mixtures, (○)GELL/LM-1 mixtures, (△)GELL/LM-2 mixtures.

V-1 The Effects of Salts on the Interaction between Gellan gum and Konjac Glucomannan

MATERIAL AND METHODS

Purified sodium form gellan sample used in this chapter was the same as described in Chapter I.

In this work, a fraction of KGM with medium molecular weight (LM-1), prepared by enzymatic degradation, was chosen among three KGM samples used in Chapter IV. The molecular weight of the sample fraction was 9.5×10^5(LM-1).

Salts, NaCl, KCl, $CaCl_2$ and $MgCl_2$ used in this study were of the extra fine grade reagents (Wako Pure Chemical Industries Ltd., Osaka, Japan), and were used without further purification. The solutions of GELL/KGM were prepared in the same way as described in section 2 of Chapter IV.

The total polysaccharide concentration was fixed at 0.8% (w/w) and mixing ratio of GELL/KGM=0.3/0.5 was chosen in the present work because the synergistic interaction was found at this ratio. For samples containing salts, the concentration of NaCl or KCl in solutions varied from 10 to 75mM and that of $CaCl_2$ or $MgCl_2$ ranged from 0.85 to 6.4mM, and each salt was added to solutions of GELL/KGM during stirring to swell before heating. Methods of rheological and DSC measurements were described in section 2 of Chapter I.

RESULTS

Rheological properties

Figure V-1(a)~(d) shows the frequency dependence of G' and G'' for GELL/LM-1 mixtures (total polysaccharide concentration 0.8%) with various mixing ratios in the absence of cations at various temperatures. At any temperature from 30℃ to 0℃, G'' for a solution of 0.8% GELL alone [Figure V-1(a)] was strongly frequency dependent, however, G' was too small to be detected. Thus, this indicated that GELL would not gel at this concentration. For a mixture with GELL/LM-1=0.4/0.4 [Figure V-1(b)], although G' could be detected at 15℃ and 0℃, these viscoelastic behaviours were typical of dilute polymer solutions. For a mixture with GELL/LM-1=0.3/0.5 [Figure V-1(c)], the viscoelastic behaviour at 30, 25 or 15℃

remained typical of a dilute polymer solution, however, the behaviour at 0℃ was quite different; G' was larger than G'' and both moduli were little frequency-dependent throughout the accessible frequency range. This behaviour is classified rheologically as that of a weak gel. However, for mixtures containing further high content of LM-1, the viscoelastic behaviour at any temperature from 30℃ to 0℃ was typical of a dilute solution. and a solution of 0.8% LM-1 alone [Figure V-2(d)] also behaved as a dilute polymer solution at any temperature. It was clear in this figure that the viscoelastic behaviour of a mixture with GELL/LM-1=0.3:0.5 tended towards that of a weak gel only at 0℃, and all other solutions behaved as dilute polymer solutions at any temperature.

Figure V-2 shows the storage modulus G' for GELL/LM-1 mixtures (total polysaccharide concentration 0.8%) in the absence of cations as a function of mixing ratio at 0.1rad/s and at various temperatures. As described in section 2 of Chapter Ⅳ, the dependence of G' on the mixing ratio was determined by values of G' at a constant frequency of 0.1rad/s in frequency sweep measurements (10^{-2}~10^{1}rad/s) at various temperatures. G' at 30, 25 or 15℃ increased continuously with increasing LM-1 content. However, G' at 0℃ showed a maximum at a mixing ratio of GELL/LM-1=0.3:0.5.

As illustrated in Figure V-1~V-2, the synergism occurred only at 0℃ at a mixing ratio of GELL/LM-1=0.3/0.5. For GELL/KGM mixtures of total polysaccharide concentration 1.6% (described in section 2 of Chapter Ⅳ), G' showed a maximum at the same mixing ratio of GELL/LM-1=0.6/1.0 at < 15℃, although the occurrence of synergism shifted to higher temperatures with increasing concentration of total polysaccharides.

Since a mixing ratio of GELL/LM-1=0.3/0.5 corresponds to the maximum of synergistic interaction for mixtures (total polysaccharide concentration 0.8%), the following experiments to study the effects of various cations were performed using mixtures of this mixing ratio.

Figure V-3(a)~(c) shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating process at the scan rate of 0.5℃/min for solutions of 0.8% GELL alone, mixture with GELL/LM-1=0.3/0.5 or 0.8% LM-1 alone in the absence of cations. For both a solution of 0.8% GELL alone and a mixture, the loss shear modulus G'' showed a step-like change at a certain temperature. As described in section 2 of Chapter Ⅰ, since this transition was attributed to the helix-coil transition in GELL molecules, the midpoint temperature of the transition T_M is defined as the temperature at which G' changes most steeply, and the relaxational strength ΔG is defined as the difference between G'' at lower and higher temperature sides at T_M measured by the ordinate unit. As described in section 2 of Chapter I, T_M shifted to lower temperatures and ΔG decreased monotonically with increasing content of KGM, and a similar tendency was observed in Figure V-3(a)~(c).

Figure V-4(a)~(c) shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating process at the scan rate of 0.5℃/min for mixtures with GELL/LM-1=0.3/0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentrations. Upon addition of 20mM NaCl [Figure V-4(a)], T_M markedly shifted to higher temperatures, although ΔG for a mixture with 20mM NaCl was not changed so much. Upon addition of 50mM NaCl [Figure V-4(b)], T_M shifted to higher temperatures, moreover, $\tan\delta = G''/G'$ became small. Upon addition of 75mM NaCl [Figure V-4(c)], the thermal behaviour of G' and G'' was quite different; both moduli could be detected at 50℃ in the cooling process and began to increase rapidly at~40 ℃ and then the values of G' were markedly larger than those of G'' at<the cross-over temperature, however, both moduli showed no drastic change up to 50℃ in heating process. Upon more progressive addition of NaCl, firm gels were formed, so that it was difficult to execute experiments. For a solution of 0.3% GELL alone (in the absence of 0.5% KGM) upon addition of 20mM, 50mM or 75mM NaCl, in all cases, G' was too small to be detected, which indicates that a solution of 0.3% GELL alone even in the presence of sufficient NaCl could not form gels (data not shown). In other words, at lower concentration of gellan gum, the number of helices formed on cooling is not sufficient to prevail the whole space and to form a three dimensional network. Therefore, although the addition of KGM was necessary for a 0.3% GELL solution to reach a gel state, the sufficient NaCl seems to promote not only the formation and aggregation of helices in GELL molecules but also the interaction between GELL and KGM, so that the mixtures in the presence of 75mM NaCl form an elastic gel with a larger thermal hysteresis. The effect of KCl on viscoelasticity of mixtures was almost similar to that of NaCl,

however, KCl influences the viscoelastic behaviour for mixtures more effectively than NaCl.

Figure V-5(a)~(g) shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating process at the scan rate of 0.5℃/min for mixtures with GELL/LM-1=0.3/0.5 (total polysaccharide concentration 0.8%) containing $CaCl_2$ of various concentrations. With progressive addition of salts, the sequence of thermal behaviour for mixtures with $CaCl_2$ was quite different from that with NaCl as shown above. Upon addition of 0.85mM $CaCl_2$ [Figure V-5(a)], the temperatures at which G' and G'' began to change in both cooling and heating processes shifted to higher temperetures than those for a mixture without salt, which was similar to results with 20, 50mM NaCl. Upon addition of 1.7mM $CaCl_2$ [Figure V-5(b)], G' and G'' in cooling process could be detected from 50℃ and increased rapidly around 30℃ and then G' became significantly larger than G'' down to 5℃, however, both moduli in heating process showed no remarkable decrease up to 50℃. This behaviour was similar to that in the presence of 75mM NaCl, however, in the presence of 1.7mM $CaCl_2$, the temperature at which both moduli change drastically was around 30℃ and this temperature was significantly lower than that in the presence of monovalent cations, and moreover the maximum values of both moduli were significantly lower than those in the presence of monovalent cations. A mixture with 1.7mM $CaCl_2$ forms firm gels on cooling to below the setting temperature and then becomes more heat resistant. Upon addition of 2.38mM [Figure V-5(c)] or 2.72mM $CaCl_2$ [Figure V-5(d)], both moduli in heating also showed no remarkable change up to 50℃, however the difference between G' or G'' in the cooling and heating process at higher temperature sides gradually decreased with increasing concentration of $CaCl_2$ from 1.7 to 2.72mM. However, in the case of more addition of $CaCl_2$ [Figure V-5(e)], the curves of G' and G'' were quite different; upon addition of 3.06mM $CaCl_2$, G'' became significantly larger than G', moreover, values of both moduli for a mixture with 3.06mM $CaCl_2$ were significantly smaller than those with lower concentration of $CaCl_2$. However, both moduli upon addition of 3.06mM $CaCl_2$ became less temperatrue dependent, and the drastic change of G'' was not clearly shown in both cooling and heating process. Upon addition of 3.4mM

$CaCl_2$ (Figure V-5(f)), although G' increased rapidly at~31℃ in the cooling process and decreased rapidly at~44℃ in the heating process, the transition at which G'' drastically changed was not observed at temperature range between 50℃ and 5℃, similarly to mixture upon addition of 3.06mM $CaCl_2$. Upon addition of 6.8mM $CaCl_2$ [Figure V-5(g)], the drastic change of G'' was not observed in both cooling and heating precess, and the temperature at which G' begins to be detected in cooling process and the temperature at which G' becomes too small to be detected in heating process shifted to lower temperatures than those with 3.4mM $CaCl_2$. Thus, in the presence of $CaCl_2$, both moduli for mixtures showed maximum at 1.7mM $CaCl_2$ and then decreased with increasing concentration of $CaCl_2$, moreover, for mixtures containing>3.06mM $CaCl_2$, the transition at which G'' drastically changed was not clearly observed in both cooling and heating processes. As illustrated in Chapter II, 1% GELL solutions in the presence of divalent cations formed an elastic gel at a higher temperature as 30℃, which indicated that the formation and aggregation of GELL molecules were promoted by the addition of sufficient divalent cation. However, for 0.3% GELL solutions in the presence of $CaCl_2$, in all cases, G' was too small to be detected (data not shown), which indicated that 0.3% GELL solutions containing $CaCl_2$ could not produce enough aggregates to reach the gel state. As shown in this figure, G' and G'' for mixtures markedly increased by addition of 1.7mM $CaCl_2$, however, both moduli significantly decreased by addition of excessive divalent cations (3.06mM, 3.4mM or 6.8mM $CaCl_2$). Although the addition of KGM was necessary for a 0.3% gellan gum solution to reach gel state, it was suggested that in the presence of sufficient $CaCl_2$, the synergistic interaction between GELL and KGM may decrease, so that G' and G'' for mixtures with sufficient $CaCl_2$ markedly decreased. The changes of temperature dependence of both moduli for mixture with increasing concentration of $MgCl_2$ were similar to those with increasing concentration of $CaCl_2$. However, G' and G'' for a mixture with 2.72mM $MgCl_2$ showed a maximum, and for mixtures containing>3.4mM $MgCl_2$, the transition at which G'' drastically changed was not clearly observed in both cooling and heating processes. These concentrations were higher than those of $CaCl_2$.

Figure V-6 shows the dependence of the midpoint temperature T_M (a) and the relaxational strength ΔG (b) for mixtures with GELL/LM-1=0.3/0.5 (total polysaccharide concentration 0.8%) on the concentration of NaCl, compared to those for solutions of 0.8% or 0.3% GELL alone. In all the three cases, T_M shifted to higher temperatures with increasing concentration of NaCl. T_M for mixtures was significantly higher than that for solutions of 0.3% GELL alone in the presence of the same concentration of NaCl. T_M for mixtures was lower than that for solutions of 0.8% GELL alone in all cases, however, the difference between T_M for 0.8% GELL solutions and T_M for mixtures in the presence of the same concentration of NaCl significantly decreased with increasing concentration of NaCl. Values of ΔG for mixtures and a solution of 0.8% GELL alone were significantly increased with increasing concentration of NaCl, however ΔG for a solution of 0.3% GELL alone only slightly increased up to 50mM NaCl and then levelled off. ΔG for mixtures was larger than that for 0.3% GELL alone at all added concentration of NaCl, especially, the difference between ΔG for mixtures and ΔG for 0.3% GELL alone markedly increased in the presence of 75mM NaCl. As described in section 2 of Chapter I, the main contribution to the magnitude of ΔG is by the formation and the subsequent aggregation of helices of GELL molecules, and this process was inhibited by the presence of KGM because ΔG for gellan alone was larger than that for mixtures at all mixing ratios. However, in the presence of sufficient monovalent cations, the formation and aggregation of helices in GELL molecules seemed to be promoted by the presence of KGM, because in the presence of 75mM NaCl, ΔG for a mixture became significantly larger than that for a solution of 0.3% GELL alone in the presence of the same concentration of NaCl.

Figure V-7 shows the dependence of the midpoint temperature T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/LM-1=0.3/0.5 (total polysaccharide concentration 0.8%) on the concentration of $CaCl_2$, compared to those for solutions of 0.8% or 0.3% GELL alone. In all the three cases, T_M shifted to higher temperatures with increasing concentration of $CaCl_2$. Although T_M for solutions of 0.8% GELL alone was significantly larger than that for solutions of mixtures or 0.3% GELL alone at the same concentration of $CaCl_2$, in the presence of 6.8mM $CaCl_2$, T_M for a solution of 0.3% GELL showed similar to that for a solution of 0.8% GELL. Although T_M for mixtures almost coincided with that for solutions of 0.3% GELL alone at lower concentrations of $CaCl_2$, T_M for mixtures levelled off around>1.7mM $CaCl_2$, so that the difference between T_M for a solution of 0.3% GELL alone and T_M for a mixture in the presence of the same concentration of $CaCl_2$ increased with increasing concentration of $CaCl_2$. ΔG for a solution of 0.8% GELL alone gradually increased up to 4mM $CaCl_2$ and then decreased with increasing concentration of $CaCl_2$. For both mixtures and solutions of 0.3% GELL alone, ΔG passed through a maximum at 1.7mM $CaCl_2$ and then decreased with increasing concentration of $CaCl_2$, The maximum value of ΔG for a mixture was significantly larger than that for solutions of 0.3% GELL alone, however, in the presence of sufficient $CaCl_2$, ΔG for mixtures was significantly smaller than that for solutions of 0.3% GELL alone.

Figure V-8(a)~(c) shows the frequency dependence of G' and G'' for mixtures with GELL/LM-1-0.3:0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentrations and at various temperatures. Upon addition of 20mM [Figure V-8(a)] or 50mM NaCl [Figure V-8(b)], the viscoelastic behaviour for a mixture was typical of a dilute polymer solution at any temperature from 30℃ to 0℃. At 30, 25, 15℃, both moduli for mixtures in the presence of 20mM or 50mM NaCl were slightly larger than those for mixtures in the absence of cations [Figure V-1(f)], however both moduli at 0℃ were significantly smaller than those for mixtures in the absence of cations. Upon addition of 75mM NaCl [Figure V-8(c)], the viscoelastic behaviour for a mixture was quite different: at any temperature from 30℃ to 0℃, particularly at a higher temperature such as 30℃, the behaviour tended towards that of an elastic gel, so that a sufficient number of junction zones are formed in a mixture by addition of sufficient NaCl. At above 45℃, G' for a mixture with 75mM NaCl could not be detected and G'' was strongly frequency dependent (data not shown), which indicated that a mixture with 75mM NaCl could not form gels at>45℃. Therefore, although the helix-coil transition temperature of GELL molecules shifted to higher temperatures by the addition of cations, in the presence of sufficient monovalent cations, the sol-gel transition temperature for GELL/KGM mixtures significatly shifted to higher temperatures.

Figure V-9(a)~(d) shows the frequency dependence of G' and G'' for mixtures with GELL/LM-1-0.3:0.5 (total polysaccharide concentration 0.8%) containing $CaCl_2$ of various concentrations and at various temperatures. Upon addition of 0.85mM $CaCl_2$ [Figure V-9(a)], the viscoelastic behaviour for a mixture was typical of a dilute polymer solution at any temperature from 30 ℃ to 0℃. At 30, 25 15℃, both moduli for mixtures in the presence of 0.85mM $CaCl_2$ were slightly larger than those for mixtures in the absence of cations [Figure V-1(c)], however both moduli at 0℃ were significantly smaller than those for mixtures in the absence of cations. This behaviour was similar to that for a mixture with 20mM NaCl [Figure V-8(a)]. Upon addition of 1.7mM $CaCl_2$ [Figure V-9(b)], the viscoelastic behaviour for mixtures at 30 ℃ remained to be that of a dilute polymer solution, however, the behaviour at a temperature lower than 25℃ tended towards that of a weak gel. However upon addition of 3.4mM [Figure V-9(c)] or 6.8mM $CaCl_2$ [Figure V-9(d)], the viscoelastic behaviour for mixtures was typical of a dilute polymer solution at any temperature from 30℃ to 0℃. Moreover, both moduli at any temperature became smaller with increasing concentration of $CaCl_2$.

Frequency dependence of both moduli for mixtures containing 1.7mM or 6.8mM $CaCl_2$ at 30℃ (similarly to Figure V-9) and 30℃ after cooling and heating cycles between 30℃ and 0℃ was shown in Figure V-10 (a) and (b) respectively. These solutions were cooled from 30℃ to 0℃ and reheated up to 30℃ at the scanning rate of 0.5℃/min, and then frequency dependence of both moduli was observed after both moduli as a function of time at 30℃ attained plateau. In the presence of 1.7mM $CaCl_2$ [Figure V-10(a)], the viscoelastic behaviour at 30℃ was typical of a dilute polymer solution, however, it changed to that of a weak gel at 30℃ after cooling and heating cycles between 30℃ and 0℃. It was suggested that the mixture with 1.7mM $CaCl_2$ which were cooled to below the setting temperature formed thermally-stable junction zones, which was in good agreement with the result in the thermal scanning rheological measurements. However, in the presence of 6.8mM $CaCl_2$ [Figure V-10(b)], both behaviours at 30℃ and 30℃ after cooling and heating cycles between 30℃ and 0℃ showed dilute polymer soluions.

Moreover, both moduli at 30℃ after cooling and heating cycles between 30℃ and 0℃ became slightly lower than those at 30℃.

DSC

Figure V-11 shows cooling and heating DSC curves for GELL/LM-1 mixtures (total polysaccharide concentration 0.8%) with various mixing ratios in the absence of cations. The cooling curve for a solution of 0.8% GELL alone [Figure V-11(a)] showed a single exothermic peak at 26.5℃, and the heating curve [Figure V-11(b)] showed a single endothermic peak at 28.0 ℃, and for a mixture of GELL/LM-1-0.7:0.1, the cooling or heating curve also showed one exothermic or one endothermic peak. However, the cooling curves for mixtures with more than LM-1 content 0.2 showed a main exothermic peak at 25~15℃ and another small peak around 95℃, while the heating curve showed a single endothermic peak. Moreover, a heating curve for a mixture of GELL/LM-1=0.5:0.3 or 0.3:0.5 showed a main exothermic peak at ~20℃ or ~15℃ and another small peaks around 84℃ and 95℃. Both main exothermic peak temperature T_s and endothermic peak temperature T_m shifted to lower temperatures and these exothermic and endothermic enthalpies for mixtures decreased with increasing content of LM-1 in mixtures, moreover, both exothermic peak and endothermic peak became very broad for mixtures with excessive LM-1. A cooling or heating DSC curve for a solution of 0.8% KGM (LM-1) alone [Figure V-11(a) and (b)] did not show any exothermic or endothermic peak, indicating that KGM alone does not show any conformational change such as helix-coil transition. However, the temperature of a small exothermic peak around 95℃ didnot change so much and this exothermic enthalpy did not change so much with increasing content LM-1 up to 0.6%. An exothermic peak at a higher temperature around 95℃ in cooling DSC curves for mixtures may be attributed to the formation of ordered structure by the interaction between GELL molecules in disordered-coil forms and KGM. This should be explored more quantitatively in the future.

Figure V-12 shows cooling and heating DSC curves for solutions of 0.3% GELL alone or mixtures with GELL/LM-1=0.3:0.5 containing NaCl of various concentrations. As is seen in this figure, in all the cases, the DSC cooling curves for both 0.3% GELL solutions and mixtures showed a main exothermic peak with T_s shifting to progressively higher temperatures withincreasing concentration of added NaCl, although many other peaks were observed both at higher and lower temperatures with increasing concentration of NaCl. In the cooling DSC curves, T_s for mixtures was significantly higher than that for solutions of 0.3% GELL alone in the presence of the same concentration of NaCl, however in the absence of salts, T_s for a mixture was slightly lower than that for solutions of 0.3% GELL alone, as will be shown later [Figure V-14(a)]. This indicated that monovalent cations promote the formation and aggregation of helices of GELL molecules rather than interaction between GELL and KGM. Although the exothermic enthalpies in cooling DSC curves for both gellan gum alone and mixtures increased with increasing concentration of NaCl, the exothermic peak for a mixture with 75mM NaCl became much sharper and this enthalpy significantly increased compared to that for a solution of GELL alone with 75mM NaCl. This result was in good agreement with the rheological result; both moduli for a mixture with 75mM NaCl significantly increased and this solution behaved as an elastic gel even at 30℃, although a solution of 0.3% GELL alone with 75mM NaCl could not form a gel at any temperature from 30℃ to 0℃. For solutions of 0.3% GELL, T_s in the heating DSC curves shifted to higher temperatures with increasing concentration of NaCl, however, upon addition of 50mM or 75mM NaCl, the heating DSC curve splitted into multiple peaks around 40~60℃, which was similar to the results as described in Chapter II. As described in Chapter II, the occurrence of detectable splitting in the heating DSC curves was observed for 1% gellan gum solutions with sufficient monovalent cations, and coincided with the occurrence of elastic gel formation at relatively higher temperatures such as 30℃. However, in the present study, the concentration of GELL investigated (0.3%) was too low to form en elastic gel even in the presence of sufficient monovalent cations, although solutions of 0.3% GELL with sufficient monovalent cation showed multiple endothermic peaks. T_m for mixtures, as well as T_s, was significantly higher than that for solutions of 0.3% GELL alone in the presence of same concentration of NaCl. T_m for mixtures shifted to higher temperatures and the heating DSC curves for mixtures became broader with increasing concentration of NaCl, however, upon addition of 75mM NaCl, the heating DSC curve for a mixture showed a main endothermic peak around 65℃, and this temperature was significantly higher than that for 0.3% GELL solution with 75mM NaCl. The fact that the main endothermic peak for a mixture with 75mM NaCl was observed around 65℃ could explain the rheological result that elastic moduli for a mixture with 75mM NaCl didnot show drastic changes during heating up to 50℃ [Figure V-4(c)]. This result indicated that KGM could shift the helix-coil transition temperature in GELL molecules to higher temperatures, and in the presence of cations, KGM could contribute to the formation of thermally-stable gels. The thermal behaviour for mixtures in the presence of KCl was similar to that for mixtures in the presence of NaCl. However, KCl influenced the thermal properties of both 0.3% GELL solutions and mixtures more effectively than NaCl. Hence, for mixture upon addition of 75mM KCl, a main exothermic peak which became much sharper was observed around 40℃ in the cooling DSC curve, while a main endothermic peak which became much broader was observed around 75℃ in the heating DSC curve with many small peaks at both lower and higher temperatures.

Figure V-13 shows cooling and heating DSC curves for 0.3% solutions of GELL alone or of mixtures with GELL/LM-1=0.3:0.5 containing $CaCl_2$ of various concentrations. In the presence of $CaCl_2$, the cooling DSC curves for both solutions of GELL alone and mixtures showed a main exothermic peak around 15~35℃ and another small peak around 95℃. Although T_s for both solutions of 0.3% GELL alone and mixtures shifted to higher temperatures with increasing concentration of $CaCl_2$, T_s for mixtures almost coincided with that for a 0.3% GELL solution in the presence of same concentration of $CaCl_2$ The endothermic enthalpies for both GELL alone and mixtures showed a maximum at 1.7mM $CaCl_2$ and then decreased with increasing concentration of $CaCl_2$. Upon addition of 6.8mM $CaCl_2$, the cooling DSC curve for a mixture became much broader and this exothermic enthalpy significantly decreased compared to that for a 0.3% GELL alone with 6.8mM $CaCl_2$. This was a similar tendency to the rheological result; elastic moduli for mixtures showed maximum at 1.7mM $CaCl_2$, although elastic moduli for mixtures were significantly larger than those for solutions of 0.3% GELL alone in all cases. Upon addition of 0.85mM $CaCl_2$, the heating DSC curve for both gellan gum alone and a mixture showed a main endothermic peak around 25℃, however, the endothermic enthalpy for a mixture with 0.85mM $CaCl_2$ was significantly smaller than that for a 0.3% GELL solution with 0.85mM $CaCl_2$ and other endothermic peaks were observed at much higher temperatures. For both 0.3% GELL solution and mixture with 1.36mM $CaCl_2$, many small peaks were observed at both lower and higher temperatures and the largest peak was observed around 102℃. With progressive addition of $CaCl_2$, the heating DSC curves for both 0.3% GELL solution and mixtures became broader and many small peaks shift to higher temperatures. As illustrated in Chapter II, although divalent cations promoted the formation of thermally stable junction zones much more strongly than monovalent cations, GELL solutions with sufficient divalent cations formed various junction zones with different thermal stabilities. Therefore, it was considered that sufficient divalent cations also made the mixtures of GELL and KGM inhomogeneous, so that the synergistic interaction between GELL and KGM molecules may be inhibited by the addition of sufficient divalent cations.

Figure V-14 shows the dependence of the exothermic peak temperature T_S (a) and the main exothermic enthalpy ΔH_S (b) for mixtures with GELL/LM-1=0.3:0.5 on the concentration of NaCl, compared to those for solutions of 0.8% or 0.3% GELL alone. In all three cases, T_S shifted to higher temperatures with increasing concentration of NaCl. Although in the absence of NaCl, T_S for solutions of 0.8% GELL alone was significantly higher than that for solutions of 0.3% GELL alone or mixture, T_S for mixtures drastically shifted to higher temperatures with increasing concentration of NaCl, eventually, T_S for a mixture with 75mM NaCl became higher rather than that for a solution of 0.8% GELL alone with 75mM NaCl. This tendency coincided with the result of T_M in the rheological measurement. Although in the absence of salts, ΔH_S for a mixture was significantly smaller than that for a solution of 0.8% or 0.3% GELL alone, ΔH_S for both 0.8% and 0.3% GELL alone didnot change so much with increasing concentration of NaCl, in contrast, ΔH_S for mixtures markedly increased with increasing concentration of NaCl. Eventually, upon addition of 75mM NaCl, ΔH_S for mixture became larger than that for not only a solution of 0.3% GELL alone but also solution of 0.8% GELL alone. These behaviours of T_S and ΔH_S induced by the concentration change of NaCl coincided with those of T_M and ΔG in the rheological measurement.

Figure V-15 shows the dependence of the exothermic peak temperature T_S (a) and the main exothermic enthalpy ΔH_S (b) for mixtures with GELL/LM-1=0.3:0.5 on the concentration of $CaCl_2$, compared to those for solutions of 0.8% or 0.3% GELL alone. In all three cases, ΔH_S increased with increasing concentration of $CaCl_2$, as well as T_S. In the absence of salt, T_S for a 0.8% GELL solution was significantly higher than that for a mixture or a 0.3% GELL solution, however, T_S for both mixtures and solutions of 0.3% GELL alone drastically increased with increasing concentration of $CaCl_2$, and upon addition of 6.8mM $CaCl_2$, these values almost coincided with T_S for a 0.8% GELL solution. Although T_S for a mixture without salt significantly was significantly lower than that for a 0.3% GELL solution without salt, in the presence of $CaCl_2$, T_S for mixtures became similar to that for solutions of 0.3% GELL alone. ΔH_S for 0.8% solutions of GELL alone didinot change so much with increasing concnetration of $CaCl_2$, however it slightly decreased by addition of >4mM $CaCl_2$. ΔHs for both mixtures and solutions of 0.3% GELL alone showed a maximum at 1.7mM $CaCl_2$ and then significantly decreased with increasing concentration of $CaCl_2$, which was in good agreement with the result of ΔG in the rheological measurement. The maximum values of ΔH_S for a mixture was slightly larger than that for a solution of 0.3% GELL alone, however, in the presence of sufficient $CaCl_2$, Δ H for mixtures was significantly smaller than that for solutions of 0.3% GELL alone.

DISCUSSION

It has been studied in many hydrocolloid mixed systems that in ternary systems containing two polymers in a common solvent, the polymers may or may not associate, as described in the introduction. Association is usually described to explain the synergistic effects observed in some of mixtures, e.g. whey proteins and carboxymethyl cellulose (Hidalgo and Hansen, 1971) or xanthan and konjac glucomannan (E.R.Morris et al., 1990b; Williams et al., 1991), whereas the segregation is thought of as a micro-phase separation, driven by an imcompatibility of the two polymers (Annable at al., 1994b; Piculell et al., 1994).

As described in section 2 of Chapter IV, the reason for the increase of G' by the addition of KGM may be ascribed to the following three possibilities: 1) An increase of effective concentration of gellan gum due to volume exclusion. 2) Segregation due to thermodynamic imcompatibility. 3) Association of gellan and KGM. If molecular association occurs between GELL and KGM, the increase in G' should be more prominent in a mixture with higher molecular weight KGM. However, as illustrated in section 2 of Chapter IV, no synergistic interaction was observed in mixtures of GELL and KGM with the highest molecular weight (ND), while mixtures of GELL and KGM with the lowest molecular weight (LM-2) show the synergistic interaction over a wider temperature range as well as over a wider mixing ratio. The elastic moduli of GELL/ND mixtures as a function of ND content increase with increasing ND content simply because G' of ND alone is far larger than that of gellan alone, so that synergism between GELL and ND should not contribute so much to the increase of G'. Therefore, in the case of GELL/ND mixtures, the first or second possibility seems to be more plausible. However, in the case of GELL/KGM with intermediate molecular weight(LM-1), G' as a function of mixing ratio showed a maximum only below 15℃ at a mixing ratio of GELL/LM-1=0.6:1.0 (total polyshaccharide concentration 1.6%). As shown in the present study, the synergistic interaction occurs only at 0℃ around GELL/LM-1 ratio 0.3/0.5 (total polysaccharide concentation 0.8%)[Figure V-1(f) and V-2], which was in good agreement with the previous result described in section 2 of Chapter IV. In the present study, solutions of 0.3% GELL alone could not form gels even if monovalent or divalent cations were present, however, a gel was formed on addition of 0.5% LM-1 at 0℃ [Figure V-1(f)]. This fact indicated that gel-like behaviour was observed when the polymers are mixed at low concentrations, where exclusion effects are likely to be negligible. It is suggested that mixed aggregates of GELL/LM-1 consist of a core of aggregates of GELL helices with KGM chains attached to the surface, as described later. A similar conclusion was reached in recent studies on gels of κ-carrageenan mixed with galactomannan or glucomannan (Williams et al., 1993; E.R.Morris, 1990b, Piculell and Lindman, 1992, Piculell et al., 1994). In this work, it seems that KGM chains attach to self-aggregates of GELL helices, rather than individual GELL helices. This reason could be explained as follows. In the cooling process, the individual helices of GELL molecules were formed at a certain temperature, corresponding to the coil-helix transition temperature, and then these helices gradually aggregated with decreasing temperature, and as result, a certain ordered structure was formed whether it could lead to the gel formation or not. As shown in the both previous and present studies, the synergism of GELL and KGM occurred at much lower temperatures than the coil-helix transition temperature. Therefore, it was suggested that the synergism should occur at low temperatures, where individual helices in GELL molecules sufficiently aggregated. This is similar to the results by Piculell et al. (1994) that the synergistic interaction disappeared in mixtures of KGM and κ-carrageenan in the presence of iodide ions, which inhibit the self-aggregation of κ-carrageenan helices. Since in the absence of cations, ΔH_S and ΔG for a mixture were slightly smaller than that for a 0.3% GELL alone [Figure V-6(b) or Figure V-7(b) and Figure V-14(b) or Figure V-15(b)], KGM molecules may hinder further aggregation of GELL helices. Upon addition of KGM, the interaction between GELL and KGM occurred so that the topological entanglement may play a role of temporary cross-linking point, however, these junction zones between KGM chains and aggregates of GELL helices should not be so stronger. In other words, the main ordered structure for mixtures was formed by aggregates of GELL helices, and KGM attached to its aggregate and formed weak junction zones. It seems that the mixed aggregates is governed by the self-aggregated GELL helices and by the mixing ratio of GELL/KGM. Upon addition of salt to mixtures, unequal partition of salt in mixtures occurred, hence, GELL-rich phase kept a higher ionic strength so that the coil-helix transition of GELL molecules in mixtures shifted to further higher temperatures. Thus, in the presence of cations, both T_M in the rheological measurement and T_S in the DSC measurement slightly shifted to higher temperatures than those for solutions of 0.3% GELL alone [Figure V-

6(a), Figure V-7(a), Figure V-14(a) and Figure V-15(a)]. Since cations can promote the aggregation of helices in gellan gum molecules by the screening effects, the introduction of cations to mixtures should permit that KGM chains attached to aggregates of GELL helices at higher temperatures. The mixed aggregates of GELL and KGM became larger with increasing concentration of cations, because the self-aggregates of GELL helices became gradually larger with increasing concentration of cations. Thus, elastic moduli for mixtures with 75mM NaCl or 1.7mM $CaCl_2$ showed larger values and these solutions formed a gel at relatively higher temperatures [Figure V-8(c) and Figure V-9 (b)]. ΔG and ΔH_S for mixtures with 75mM NaCl or 1.7mM $CaCl_2$ also became larger than those for a 0.3% GELL alone with the same concentration of cations [Figure V-6(b), Figure V-7(b), Figure V-14(b) and Figure V-15(b)]. Moreover, since in the heating process, KGM which attached to large aggregates of GELL helices could retard the helix-coil transition in GELL molecules, the melting temperature shifted to higher temperatures [Figure V-12(b) and Figure V-13(b)], hence, a larger thermal hysteresis was observed for these solutions [Figure V-4(c) and Figure V-5(b)].

In the presence of sufficient divalent cations, since GELL in mixtures could form various junction zones with different thermal stabilities, as described in Chapter II. Thus, in the presence of sufficient divalent cations, many self-aggregates of GELL helices were formed and then KGM chains could attach to each surface of its aggregate. However, since the size of each aggregate of GELL helices became smaller, the size of mixed aggregates became smaller. This may explain the fact that the cooling DSC curve for mixtures with sufficient divalent cations became much broader compared to that for solutions of 0.3% GELL alone with the same concentration of divalent cations or mixtures with lower concentration of divalent cations. G' for mixtures with sufficient divalent cations also decreased rather than that for mixtures without divalent cations [Figure V-3(b) and Figure V-5(g)] but showed a larger value than that for 0.3% GELL solutions with and without divalent cations (data not shown) because G' for LM-1 alone was far larger than that for gellan gum alone. This may indicate that polymer segregation, which leads to weaker ordered structures, occurs. This explanation is favored by the possibility that the intermolecular binding complexes between GELL and

KGM molecules could not form.

It was interesting that mixtures with low concentration of divalent or monovalent cations could not form gels even at 0℃ [Figure V-8 (a),(b) and Figure V-1-9(a)], although a mixture without salt could form weak gel only at 0℃ [Figure V-1(f)]. If KGM interacts with GELL in coil conformation, since the electrostatic shield leads to the reduction of coil dimensions, the overlap between GELL coils and KGM chains should decrease in the presence of salts, and hence synergistic interaction between GELL and KGM would decrease. However, since with more progressive addition of salt, the elastic moduli for mixtures significantly increased and a gel formation was observed, this possibility should be excluded. Although KGM was uncharged, cations changed water structures around KGM molecules or GELL molecules, so that the chances that KGM chains attach to the self-aggregated GELL helices may decrease. This simply may relate to the low total polysaccharide concentration, however, it seems to be difficult to explain this phenomenon by a single factor.

Annable et al. (1994b) have studied for mixed potato maltodextrin (PMD)/hydrocolloid systems, and have reported that in the case of mixed PMD/carboxymethyl cellulose (CMC), phase separation could not be observed at all in the absence of electrolyte for the compositions investigated but was observed when electrolyte was present. They have also found that in the case of mixed PMD/gum arabic(GA), phase separation was observed without added electrolyte but only at very high polymer concentration. Both high polymer concentration and addition of salt to such mixtures (one of polymers in the mixture carried an ionic charge) should generally result in an increase of segregation. If the concentration of gellan gum increases, where gellan gum itself could form gel whether cations are present or not, the segregation due to thermodynamic imcompatibility was promoted, so that no synergistic interaction may be observed in such high polymer concentration mixtures. This should be investigated in the near future.

We conclude that KGM is adsorbed onto the surface of large aggregates of GELL helices, and not only the gellan gum self-aggregation but also these interactions between GELL and KGM were promoted with increasing concentration of cations. Since sufficient divalent cations formed various aggregates of GELL helices with different thermal stabilities, the phasesaparation in GELL/KGM mixtures would promote in the presence of sufficient divalent cations. However, the present data were obtained only from rheological and thermal measurements, indeed, more detailed studies based on other methods to describe the phenomena at the molecular level are required.

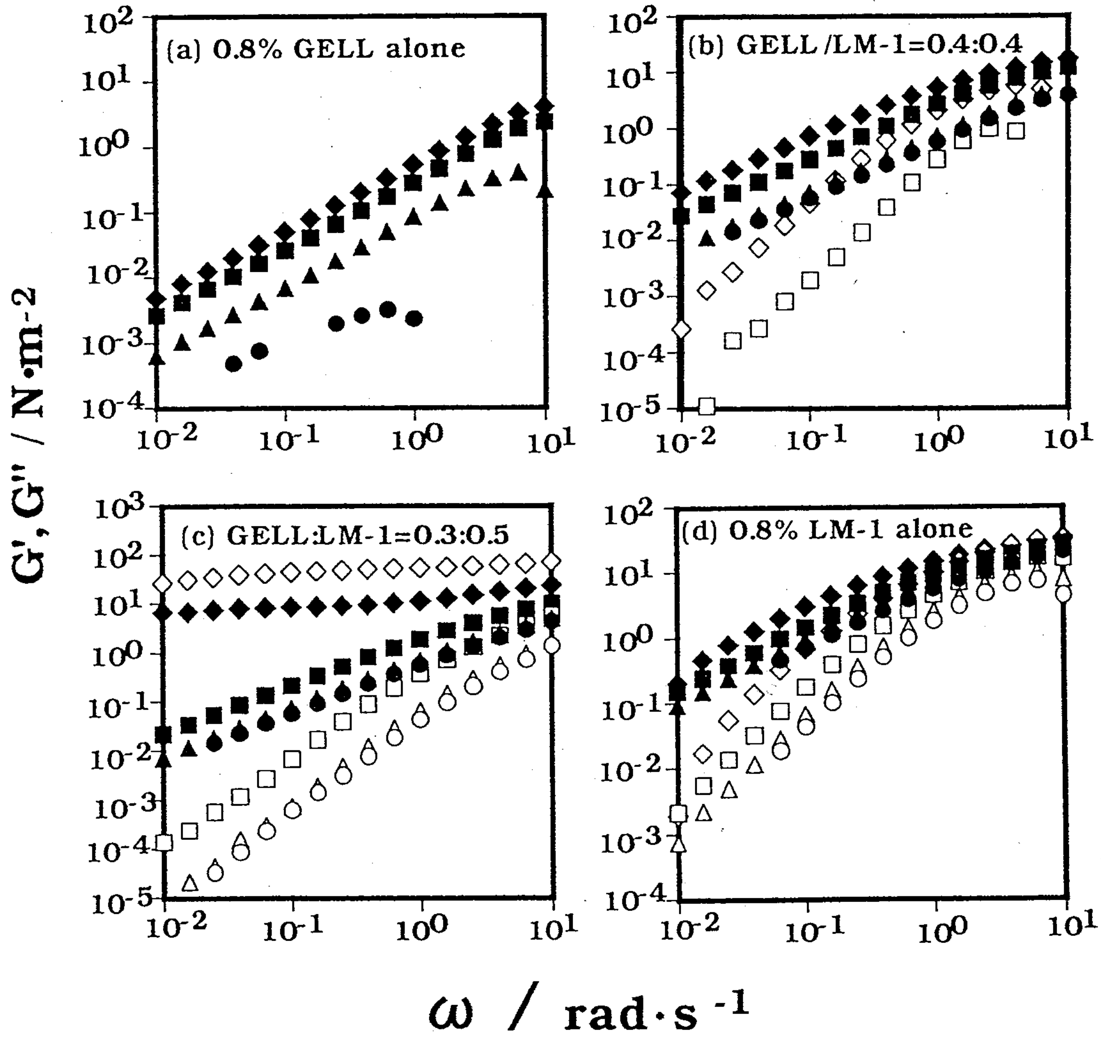

Fig. V-1 Frequency dependence of storage modulus G' and G" for GELL /LM-1 mixtures (total polysaccharide 0.8%) with various mixing ratios in the absence of cations at various temperatures. (○)G',(●)G",30℃; (△)G',(▲)G",25℃; (□)G',(■)G",15℃; (◇)G',(◆)G",0℃.

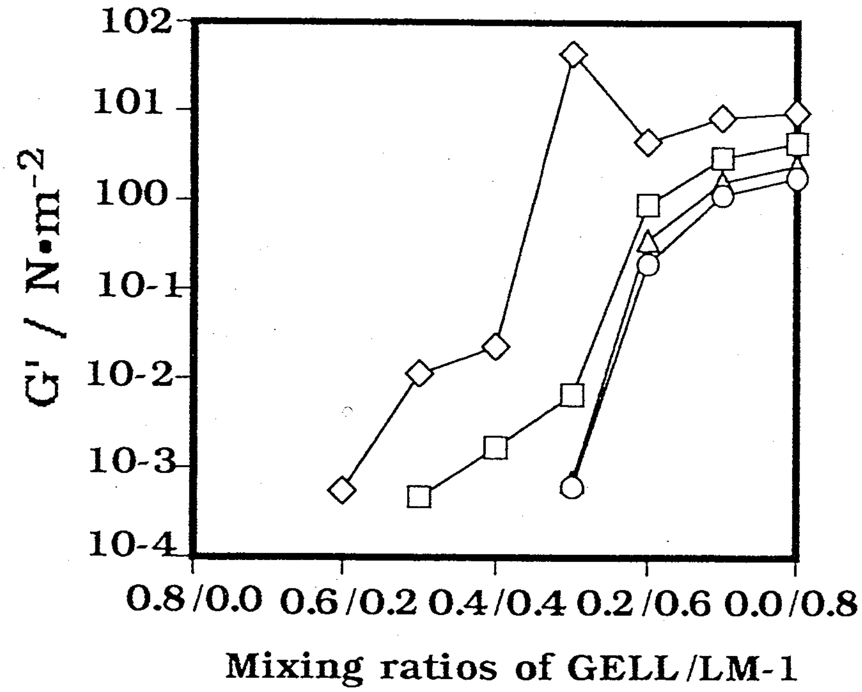

Fig. V-2 Storage modulus G' for GELL/LM-1 mixtures (total polysaccharide 0.8%) in the absence of cations as a function of mixing ratio at various temperatures. (○)30℃, (△)25℃, (□)15℃, (◇)0℃; Freq.ω=0.1rad/s.

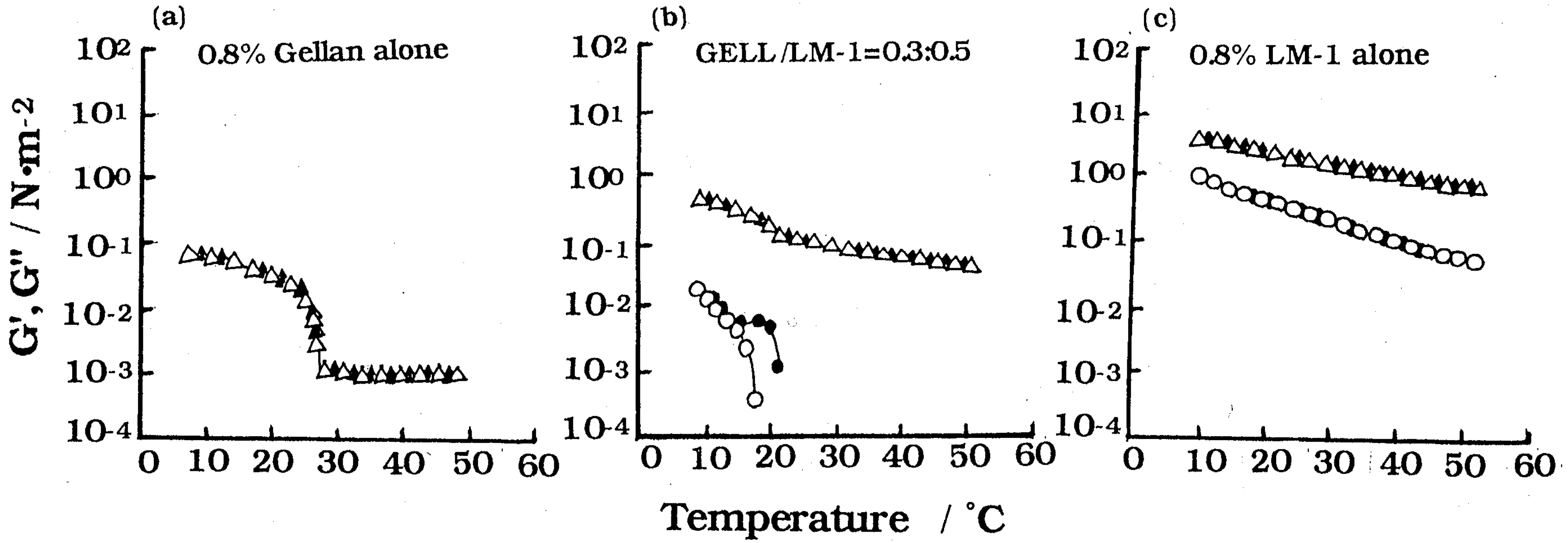

Fig. V-3 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a solution of 0.8% GELL alone (a), mixture with GELL/LM-1=0.3:0.5 (b) or solution of 0.8% LM-1 alone (c) in the absence of cations. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate: 0.5℃/min; Freq.ω=0.1rad/s.

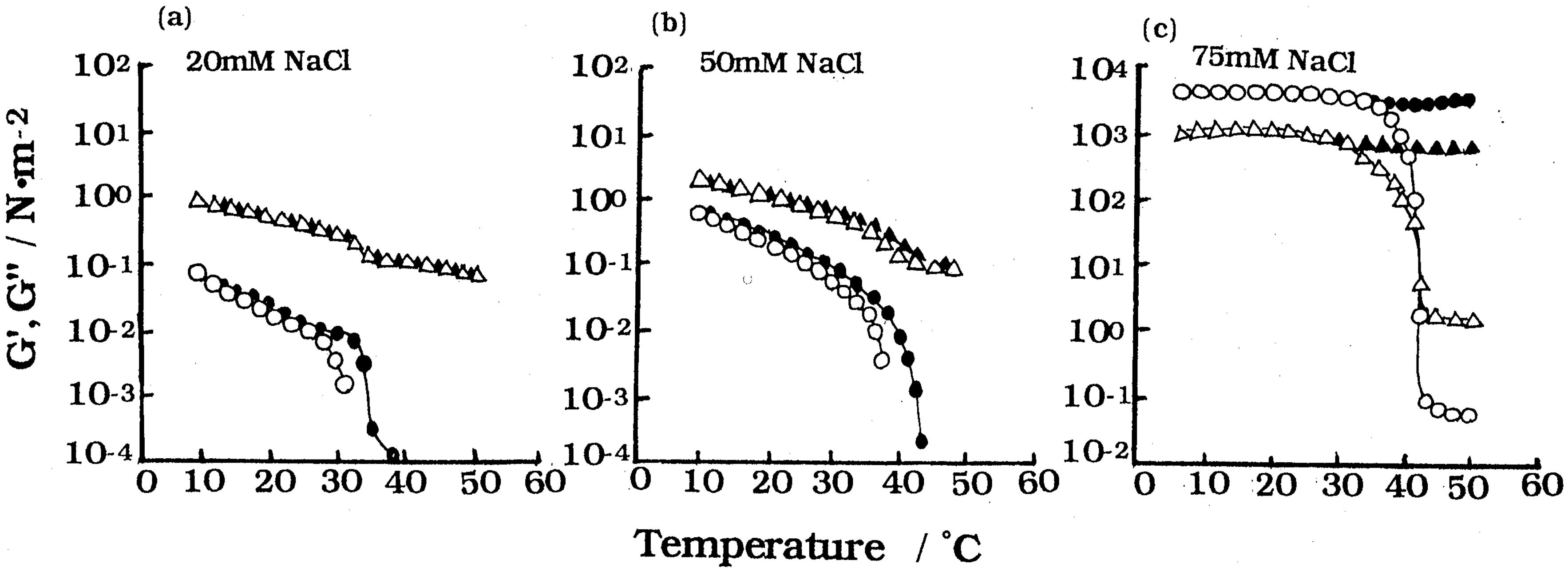

Fig. V-4 Temperature dependence of storage modulus G' and loss modulus G" during cooling and heating process for a mixture with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentration. (○)G', (△)G", cooling; (●)G', (▲)G", heating; cooling and heating rate: 0.5℃/min; Freq.ω=0.1rad/s; NaCl conc.: (a)20mM, (b)50mM, (c)75mM.

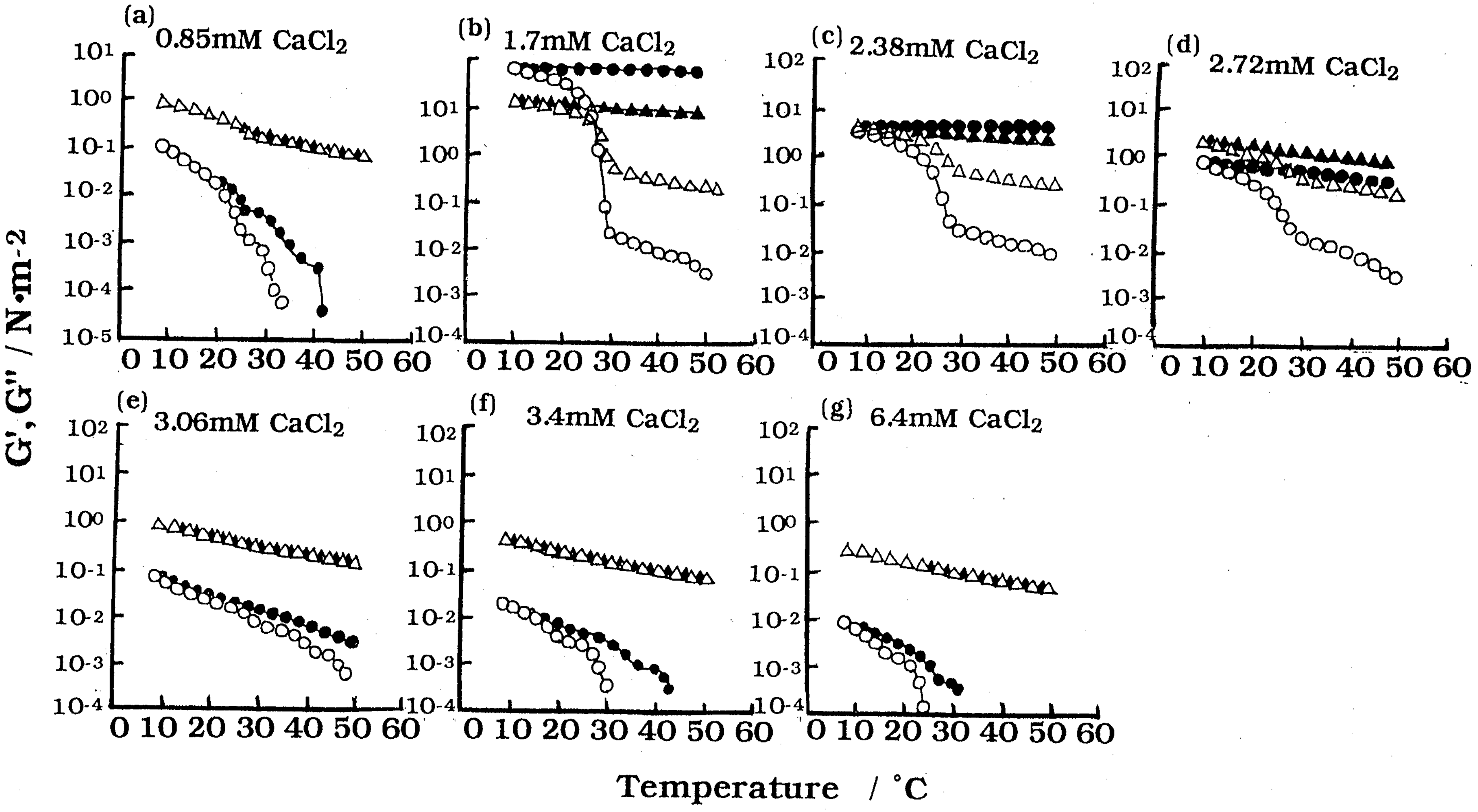

Fig. V-5 Temperature dependence of storage modulus G' and loss modulus G'' during cooling and heating process for a mixture with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%) containing CaCl2 of various concentration. (○)G', (△)G'', cooling; (●)G', (▲)G'', heating; cooling and heating rate: 0.5℃/min; Freq.ω=0.1rad/s; $CaCl_2$ conc.: (a)20mM, (b)50mM, (c)75mM.

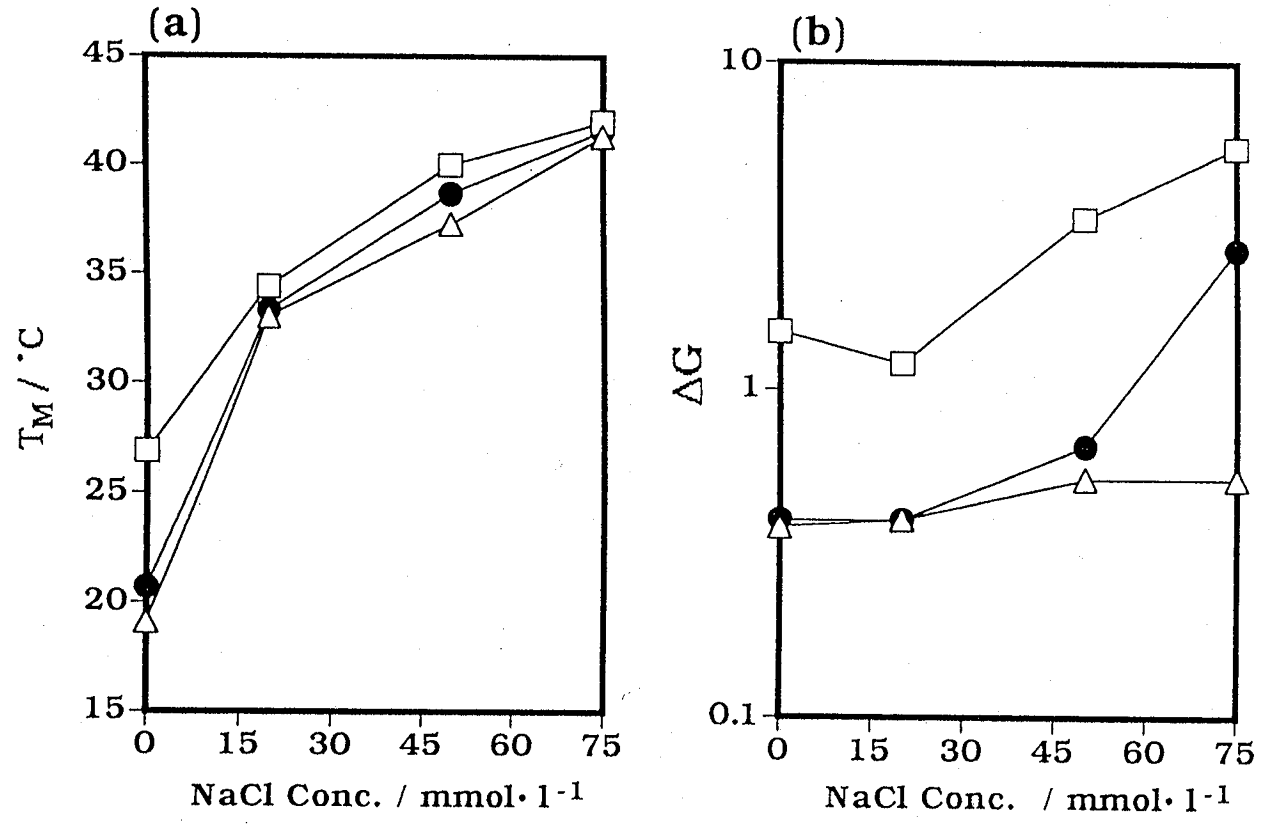

Fig. V-6 Dependence of midpoint temperature of transition T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%), 0.3% or 0.8% GELL solutions on the concentration of NaCl. (●)mixture, (△)0.3% GELL, (□)0.8% GELL.

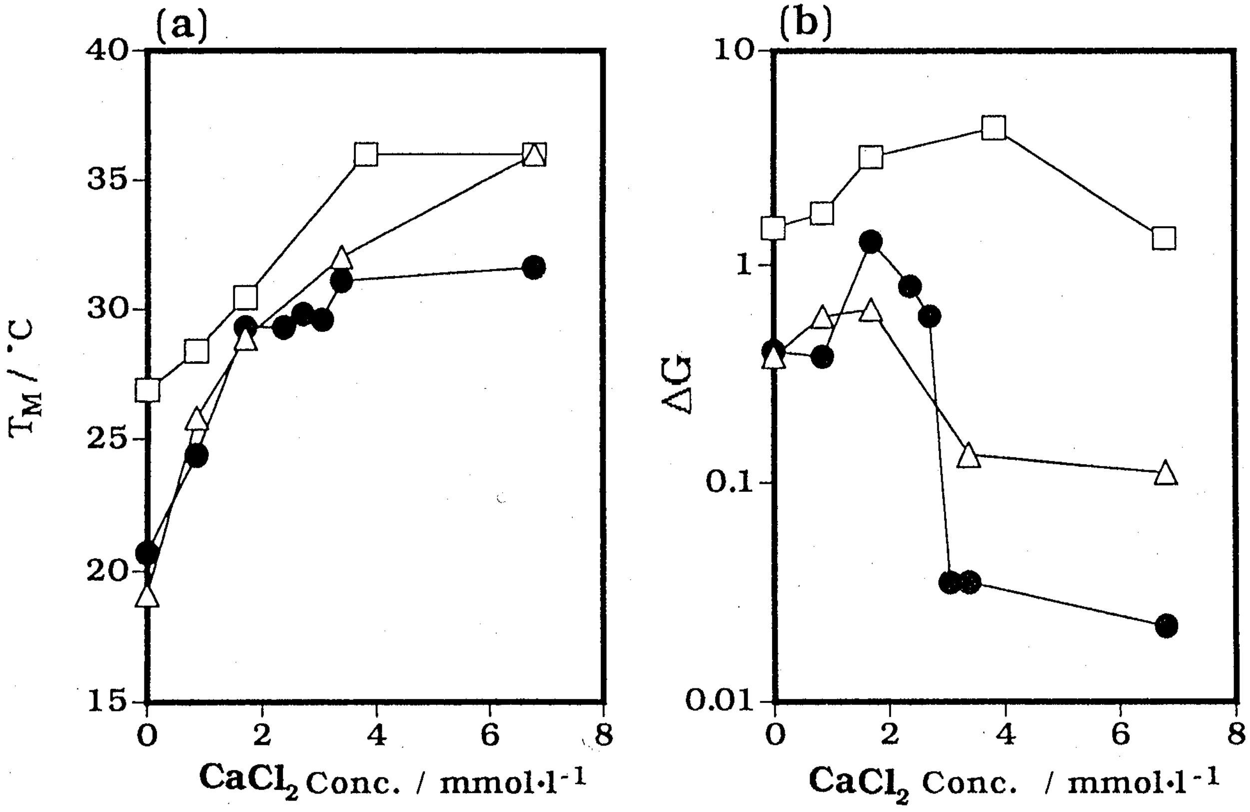

Fig. V-7 Dependence of midpoint temperature of transition T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%), 0.3% or 0.8% GELL solutions on the concentration of $CaCl_2$. (●)mixture, (△)0.3% GELL, (□)0.8% GELL.

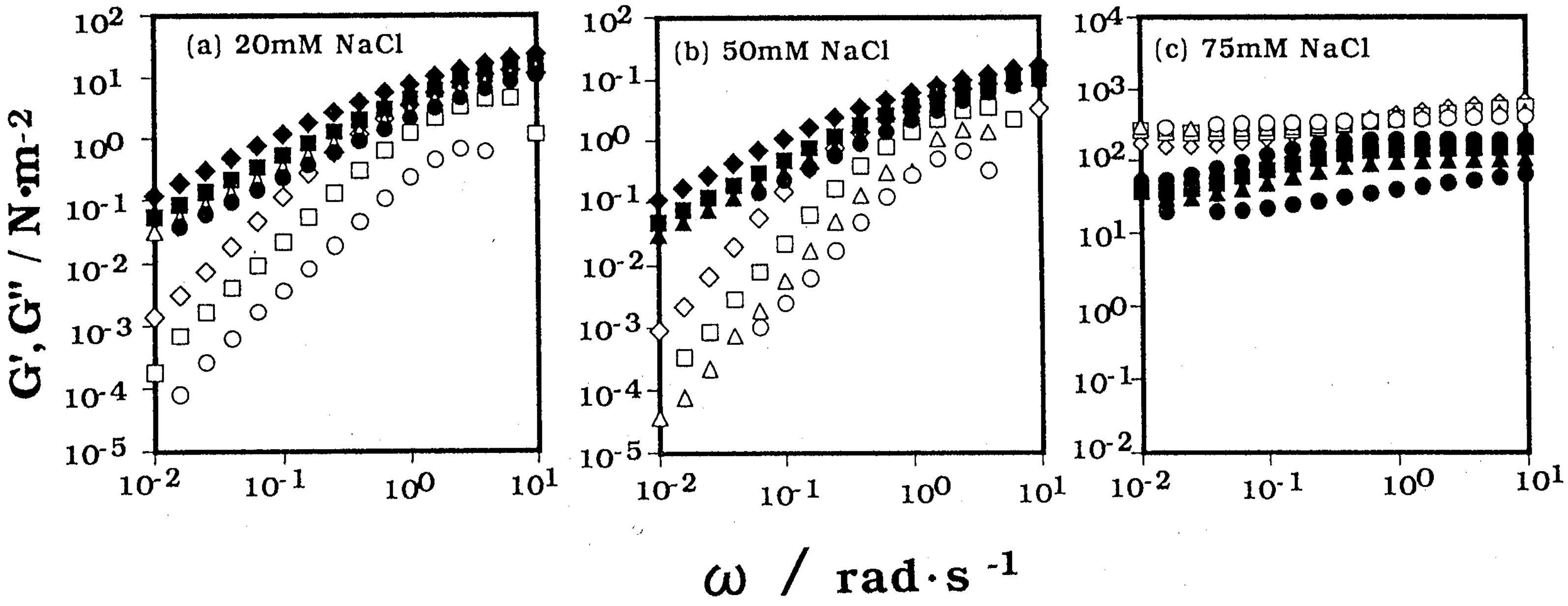

Fig. V-8 Frequency dependence of storage modulus G' and loss modulus G'' for mixtures with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentration at various temperatures. (○)G', (●)G'', 30℃; (△)G', (▲)G'', 25℃; (□)G', (■)G'', 15℃; (◇)G', (◆)G'', 0℃; NaCl conc.: (a)20mM, (b)50mM, (c)75mM.

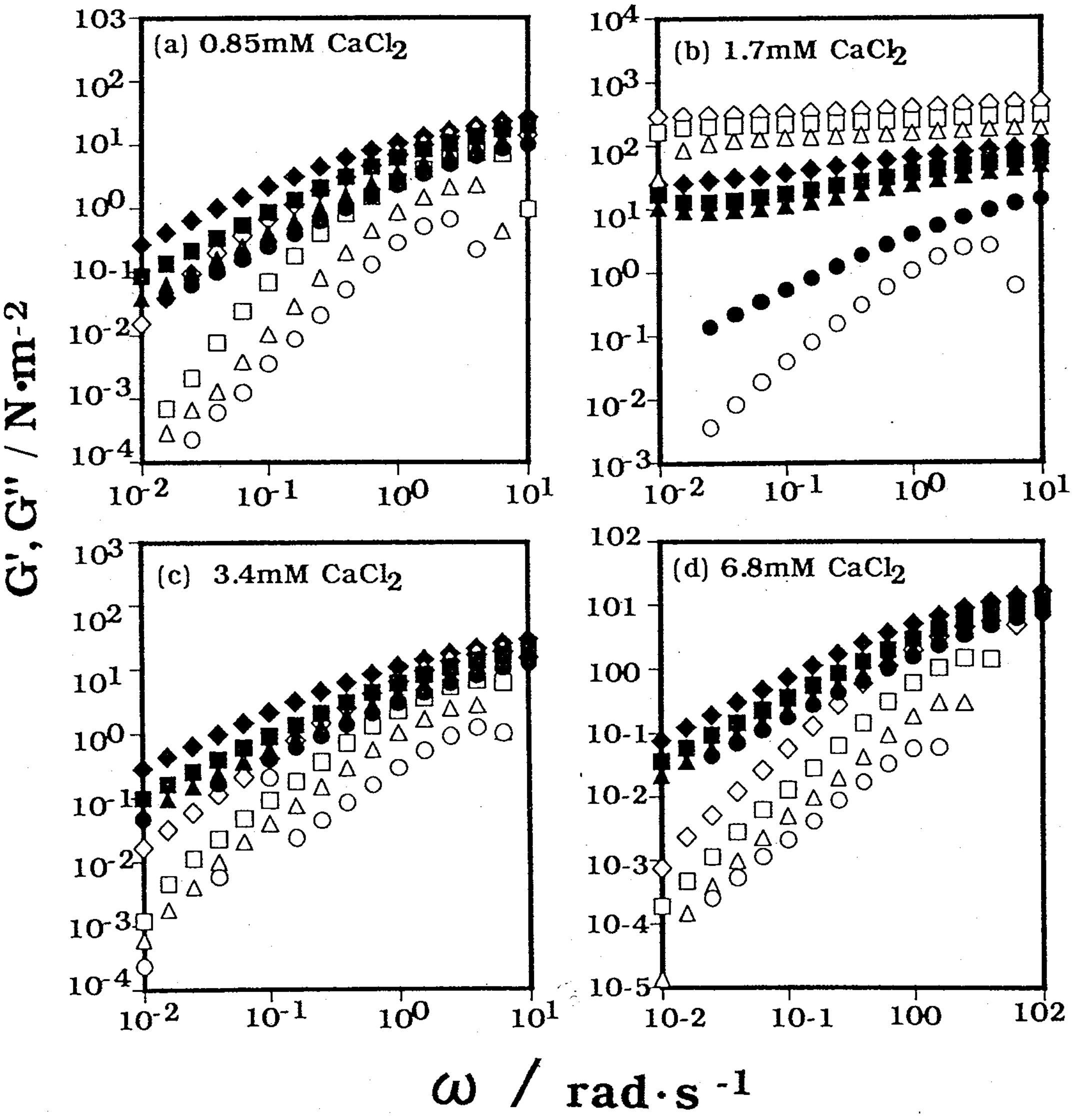

Fig. V-9 Frequency dependence of storage modulus G' and loss modulus G" for mixtures with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%) containing $CaCl_2$ of various concentration at various temperatures. (○)G',(●)G",30℃; (△)G',(▲)G",25℃; (□)G',(■)G",15℃; (◇)G',(◆)G',0℃; $CaCl_2$ conc.: (a)0.85mM, (b)1.7mM, (c)3.4mM, (d)6.8mM.

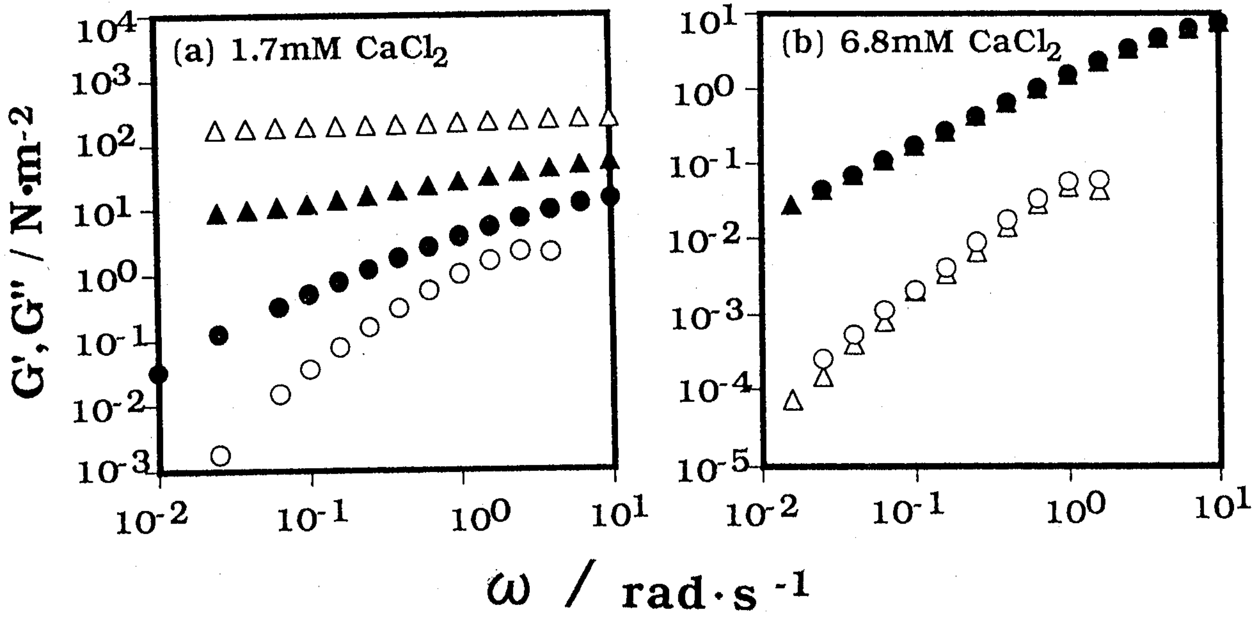

Fig. V-10 Frequency dependence of storage modulus G' and loss modulus G" for mixtures with GELL/LM-1=0.3:0.5 (total polysaccharide concentration 0.8%) containing 1.7mM or 6.8mM $CaCl_2$ at 30℃, and 30℃ after cooling and heating cycles between 30℃ and 0℃. (○)G',(●)G",30℃; (△)G',(▲)G",30℃ after cooling and heating cycles between 30℃ and 0℃; Cooling and heating rate: 0.5℃/min.

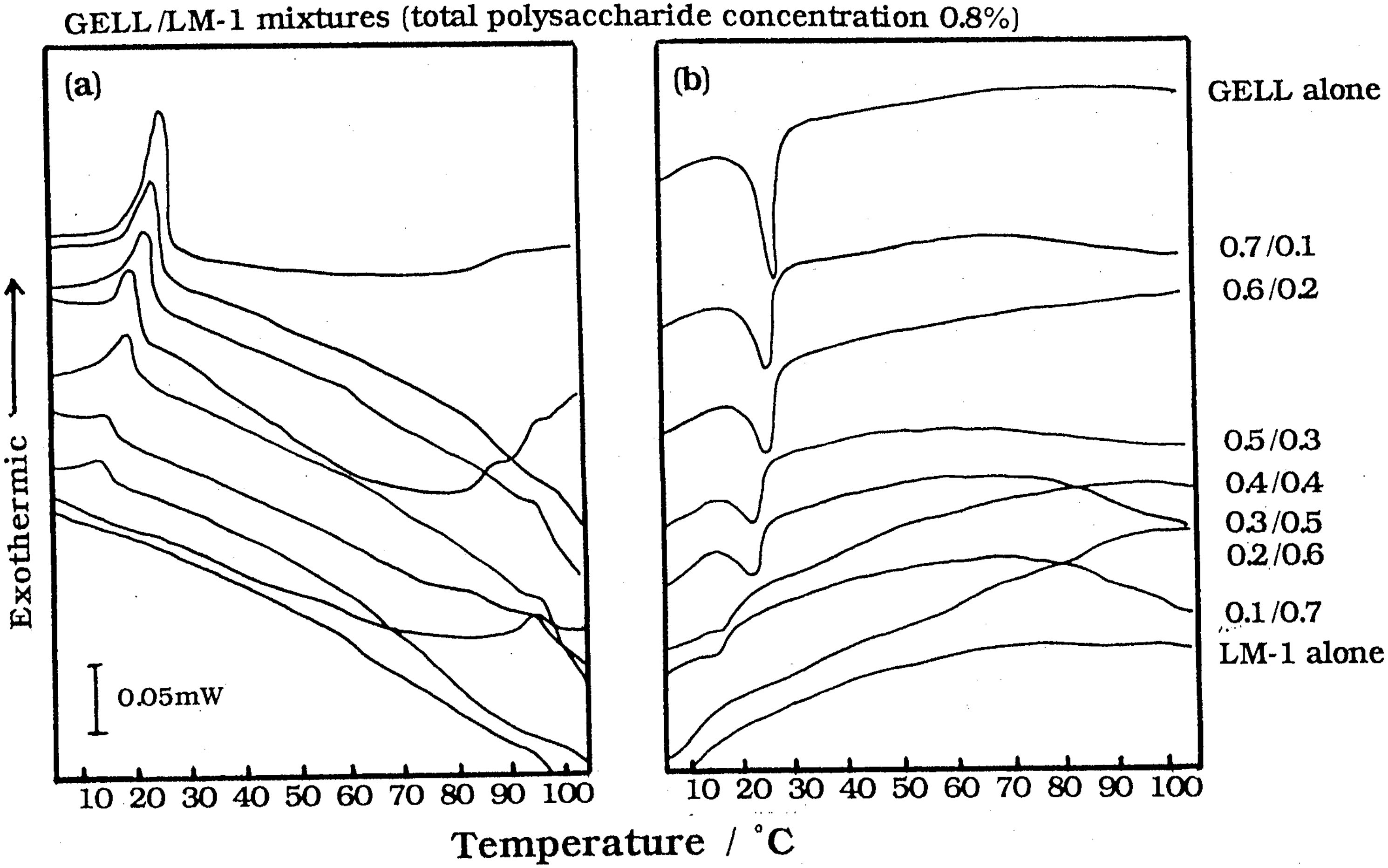

Fig. V-11 Cooling (a) and heating (b) DSC curves for GELL/LM-1 mixtures (total polysaccharide concentration 0.8%) with various mixing ratios. Cooling and heating rate: 0.5℃/min; Figures beside each curve represent the mixing ratio of GELL/LM-1.

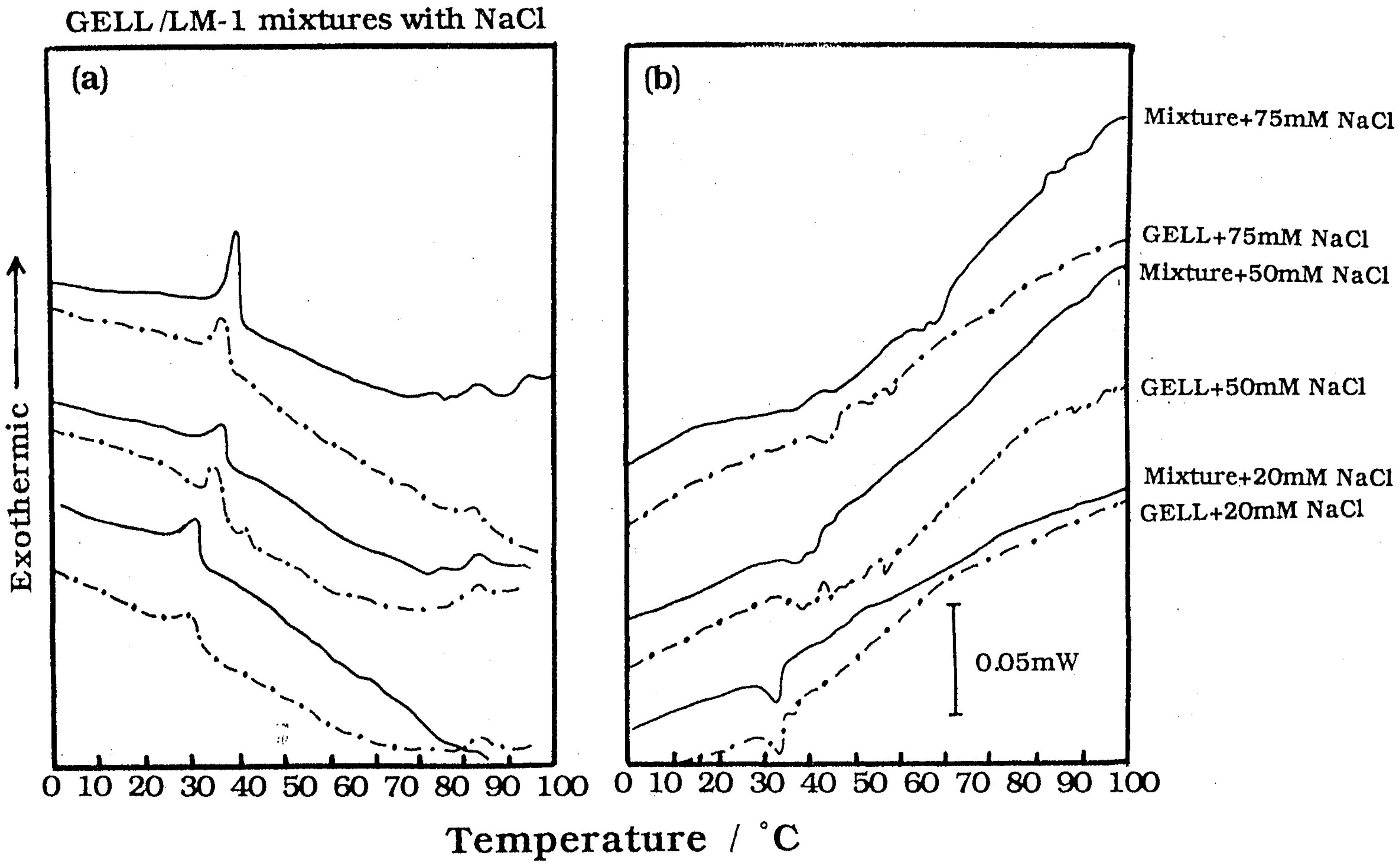

Fig. V-12 Cooling (a) and heating (b) DSC curves for mixtures with GELL/LM-1=0.3:0.5 (——) or 0.3% GELL solutions (——) containing NaCl of various concentrations. Figures besides each curve represent the concentration of NaCl.

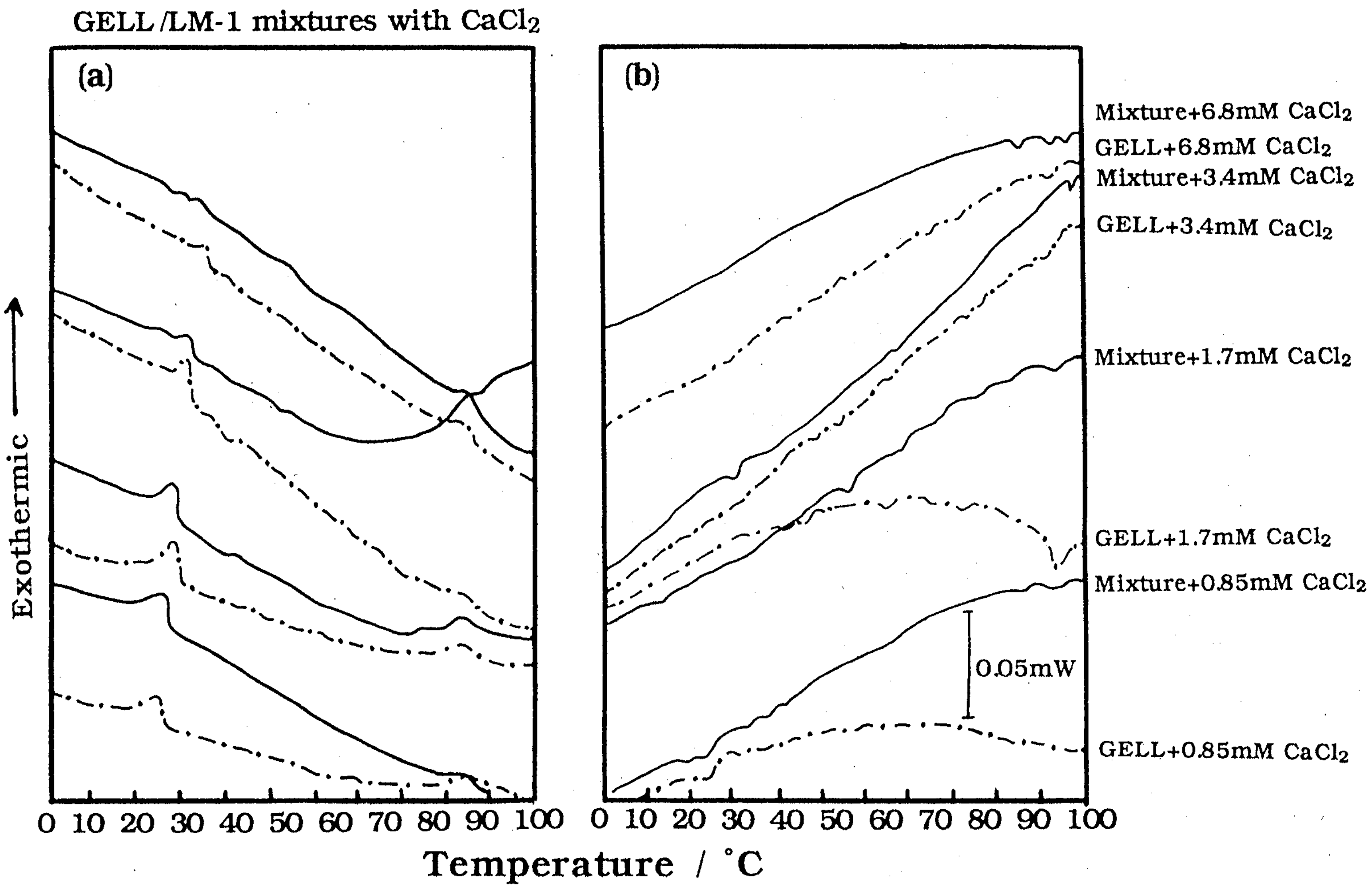

Fig. V-13 Cooling (a) and heating (b) DSC curves for mixtures with GELL/LM-1=0.3:0.5 (——) or 0.3% GELL solutions (——) containing $CaCl_2$ of various concentrations. Figures besides each curve represent the concentration of $CaCl_2$.

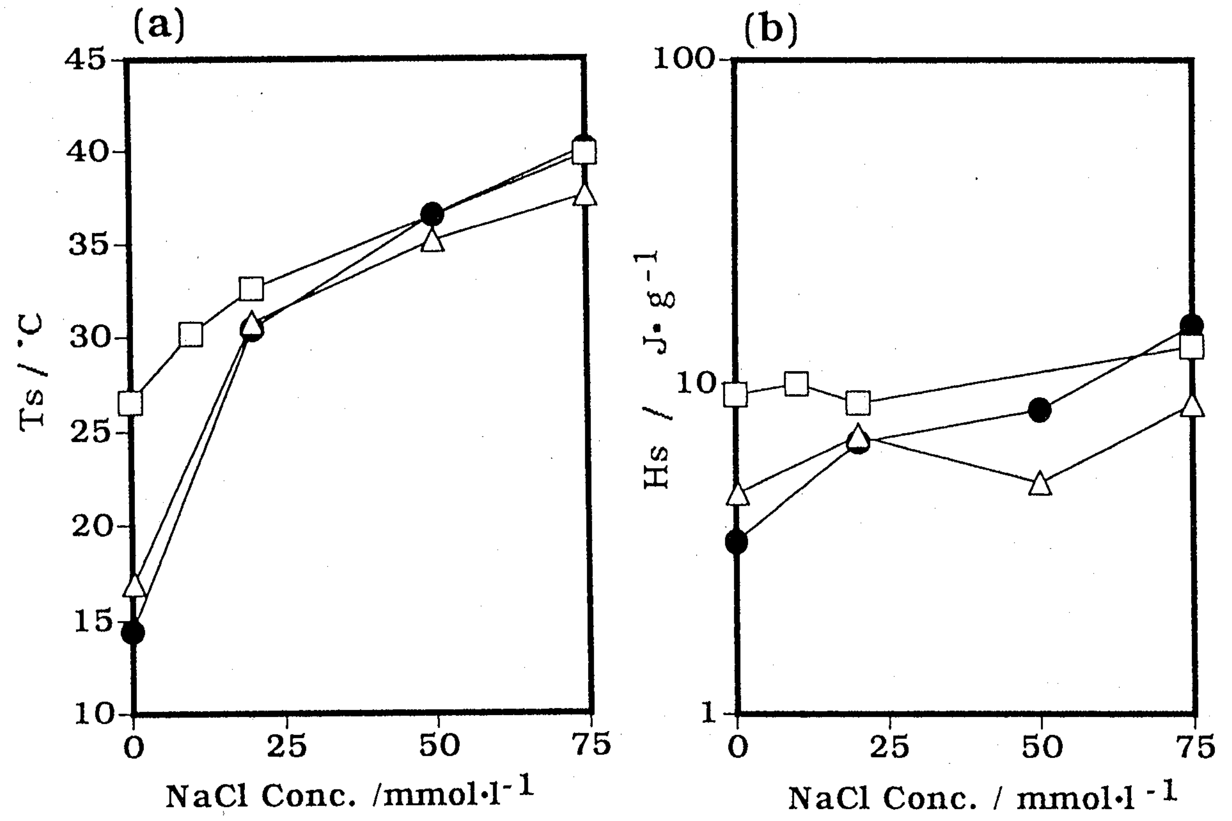

Fig. V-14 Dependence of the exothermic peak temperature T_s(a) and the exothermic enthalpy ΔH_s(b) for mixtures with GELL/LM-1 =0.3:0.5 (total polysaccharide concentration 0.8%), 0.3% or 0.8% GELL solutions on the concentration of NaCl. (●)mixture, (△)0.3% GELL, (□)0.8% GELL.

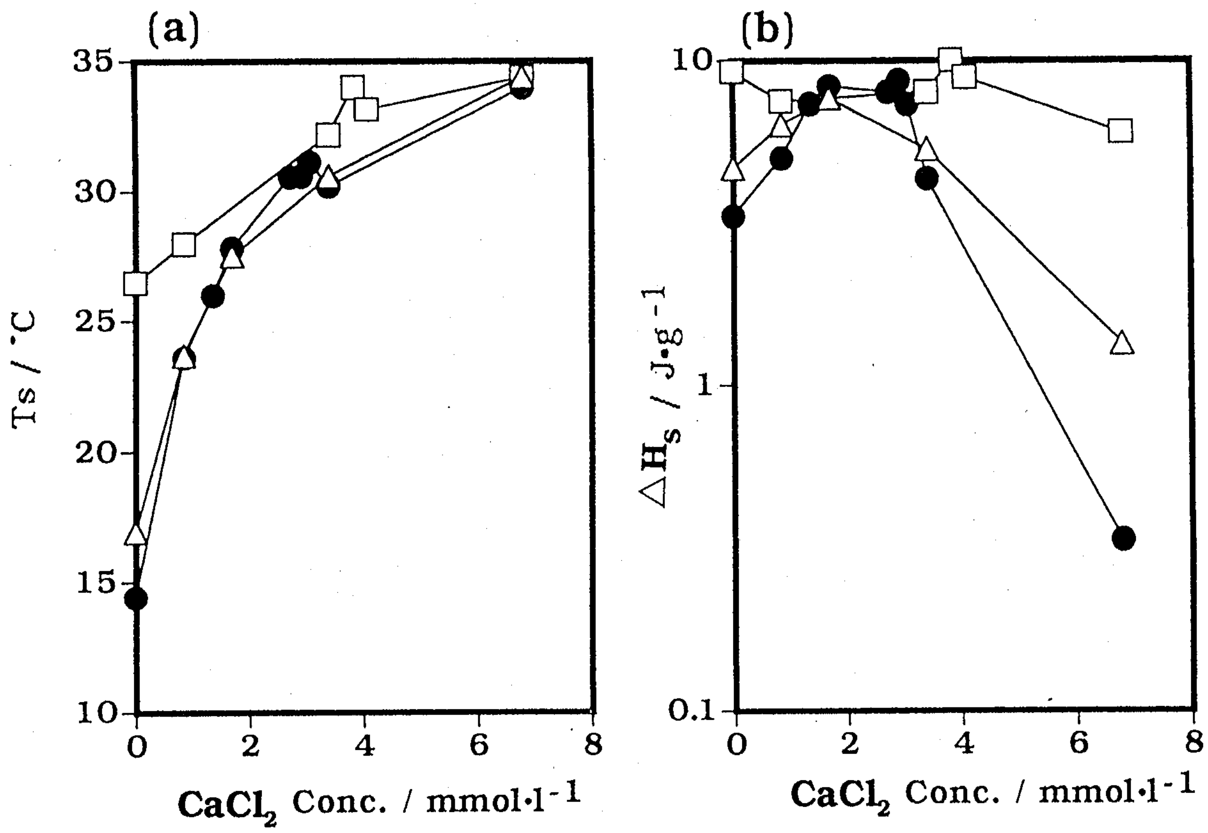

Fig. V-15 Dependence of the exothermic peak temperature T_S (a) and the exothermic enthalpy ΔH_S (b) for mixtures with GELL/LM-1 =0.3:0.5 (total polysaccharide concentration 0.8%), 0.3% or 0.8% GELL solutions on the concentration of $CaCl_2$. (●)mixture, (△)0.3% GELL, (□)0.8% GELL.

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SUMMARY

Chapter I

The rheological and thermal properties of sodium form gellan gum solutions were studied using dynamic viscoelastic measurement and differential scanning calorimetry. At any temperature from 30℃ to 0℃, the viscoelastic behaviour of a 1% gellan gum solution without salt was typical of a dilute solution, however, that of a 2% gellan gum solution varied from a dilute solution to a weak gel with decreasing temperature. The behaviour of a 3% gellan gum solution tended towards that of a weak gel, even at a relatively higher temperature as 25℃. Temperature dependence of the loss shear modulus G" for gellan gum solutions of lower concentrations without salt showed one step-like change at a certain temperature, however, that for concentrated gellan gum solutions (>2.0%) showed two step-like changes. The higher temperature process was attributed to the helix-coil transition, and was found inbetween the exothermic and endothermic peak temperatures T_s and T_m observed in cooling and heating DSC curves, while the lower temperature process was attributed to the sol-gel transition. Temperature dependence of G" for gellan gum solutions of higher concentrations (>3.2%) showed a larger hysteresis, moreover, the temperature at which G" showed the second step decrease in the heating process shifted to higher temperatures with increasing concentration of gellan gum. The cooling or heating DSC curves for gellan gum solutions of lower concentrations showed a single exothermic and endothermic enthalpy increased with increasing concentration of gellan gum. However, for a gellan gum solution of a concentration higher than 3.2%, an endothermic peak in a heating DSC curve splitted into two peaks, while the cooling curve showed only one exothermic peak. The lower temperature endothermic peak in a heating DSC curve corresponds with the first step decrease of G" in the heating process, and the higher temperature endothermic peak corresponds with the second step decrease of G".

Chapter II

The mechanical spectra and thermal properties of sodium form gellan gum solutions with and without NaCl, KCl, $CaCl_2$ and $MgCl_2$ were studied by dynamic viscoelastic measurement and differential scanning calorimetry. Storage shear modulus G' for 1% gellan gum solutions increased on addition of salts at lower temperatures where gellan molecules take a helical conformation whilst it decreased on addition of salts at higher temperatures where gellan molecules take a coil conformation. Salts promote aggregation of helices at lower temperatures whilst they reduce the coil dimension at higher temperatures. The increase in G' for gellan gum solutions by the addition of cations was attributed to the electrostatic shield of the repulsion between carboxyl groups. This effect was more remarkable for K^+ than Na^+, and for Ca^{2+} than Mg^{2+}. The DSC cooling curves showed a single exothermic peak, with the setting temperature T_s shifting to progressively higher temperatures with increasing concentration of monovalent cations. At low concentration of monovalent cations, the DSC heating curves showed a single endothermic peak, however, with more progressive addition of salt, the endothermic peak gradually developed bimodal character and eventually splitted into more than two peaks. With increasing concentration of divalent cations, the exothermic and endothermic enthalpies estimated for a main peak increased up to a certain concentration and then decreased. Moreover, the endothermic peaks with excessive salts were too broad to be resolved from the baseline. Thermal scanning rheology (temperature dependence of G' and G" at the same scan rate as DSC) showed the similar phenomena for the different effects of monovalent and divalent cations on the sol-gel transition. In the presence of sufficient divalent cations, many small endothermic peaks were observed at higher temperatures and the largest endothermic peak was observed at a temperature higher than 100℃. Therefore, although divalent cations promote the formation of thermally stable junction zones much more strongly than monovalent cations, the gellan gum gels containing divalent cations may also consist of the physical junction zones by hydrogen bonds because these junction zones can be unzipped on heating to 120℃.

Chapter III

Effects of glucose, sucrose, mannose and konjac glucomannan with different molecular weights on the sol-gel transition in gellan gum aqueous solutions were studied by dynamic viscoelastic measurement and differential scanning calorimetry. Temperature dependence of the loss shear modulus G'' for a 0.5 or 1% gellan gum solution without sugars showed a one step-like change at a certain temperature, which corresponded to the helix-coil transition temperature T_{hc}. In the presence of any sugar, storage shear modulus G' and loss shear modulus G'' increased and T_{hc} shifted to higher temperatures. The relaxational strength ΔG significantly increased by the addition of sugar. With progressive addition of sugar, the cross-over of G' and G'' was observed at a lower temperature than T_{hc}, which was attributed to the sol-gel transition T_{sg}. Moreover, in the presence of sufficient sugar, both moduli did not decrease remarkably during heating up to 60℃, although both moduli showed a drastic increase around 50℃ during cooling process. Both moduli increased by the addition of all these sugars in the order of effectiveness: sucrose>glucose>mannose. In the presence of any sugar of fairly low concentration, DSC measurement showed that both exothermic and endothermic peak temperatures, T_s and T_m, slightly shifted to lower temperatures, and that exothermic and endothermic enthalpies, ΔH_s and ΔH_m, significantly decreased. In contrast, rheological measurement showed that elastic moduli and the relaxational strength ΔG significantly increased by the addition of a sugar of fairy low concentration. However, with further addition of sugar, T_s and T_m shifted to higher temperatures and ΔH as well as elastic moduli, increased. It, therefore, seems that the addition of sugar to gellan gum solutions increases the number of junction zones, however, the size of each junction becomes small if the amount of sugar is not sufficient, so that the addition of a sugar of fairly low concentration could increase the elastic moduli but decrease the thermal stability. However, with progressive addition of sugars, small but more numerous junction zones are formed, so that not only elasticity but also thermal stability of gellan gum gels increases. In the presence of sufficient sugar, both cooling and heating DSC curves became broader, however, many small peaks were observed at both higher and lower temperatures. Gellan with sufficient sugar formed various junction zones with different thermal stabilities during cooling, and then these junction zones became markedly heat resistant. In the presence of konjac glucomannan with relatively lower molecular weight, the effective concentration of gellan gum increases by immobilising water molecules in gellan gum solutions, so that konjac glucomannan could indirectly promote the helix-coil transition in gellan gum molecules.

Chapter IV

Interaction between gellan gum (GELL) and konjac glucomannan (KGM) has been studied by dynamic viscoelastic measurements and differential scanning calorimetry (DSC). Three fractions of konjac glucomannan with different molecular weights (1.17×10^6(ND),9.5×10^5(LM-1) and 2.51×10^5(LM-2)) were used in this study. Mechanical spectra showed that 1.6% solutions of GELL alone and of LM-2 alone behaved like a dilute polymer solution whilst 1.6% solutions of ND alone and LM-1 alone behaved like a concentrated polymer solution where molecular entanglements play an important role. Thermal scanning rheological measurements showed a step-like change in the loss modulus which is attributed to the helix-coil transition, and subsequent aggregation of helices, and an exothermic and endothermic peak appeared in cooling and heating DSC curves respectively at the same temperature of transition. The transition temperature shifted to lower temperatures, and the transition enthalpy decreased with increasing KGM content in the mixture. The elasticity of mixtures originates mainly from aggregated helices of gellan gum molecules for GELL/LM-2 systems whilst the contribution from KGM is more important for GELL/LM-1 and GELL/ND systems. Although the storage modulus of GELL/LM-1 and GELL/LM-2 as a function of mixing ratio showed a maximum, it is concluded that KGM inhibits the formation of the ordered structure of gellan gum.

Chapter V

Interaction between gellan gum (GELL) and konjac glucomannan (KGM) with and without sodium chloride, potassium chloride, calcium chloride and magnesium chloride has been studied using dynamic viscoelastic measurements and differential scanning calorimetry. The mixing ratio of GELL/KGM was chosen at 0.3%:0.5% (total polysaccharide concentration 0.8%) because the synergistic interaction only at lower temperatures was found at this ratio. With progressive addition of monovalent cations, storage shear modulus G' and loss shear modulus G'' for mixtures increased, and not only the helix-coil transition temperature of GELL molecules in mixtures but also the sol-gel transition temperature for mixtures shifted to higher temperatures with increasing concentration of cations. Mixtures with sufficient monovalent cations form an elastic gel with a large thermal hysteresis. In the presence of divalent cations, G' increased up to a certain concentration and then decreased with increasing concentration of cations, although the helix-coil transition temperature of GELL molecules in mixtures monotonically increased with increasing concentration. The exothermic and endothermic peak temperatures for mixtures in the cooling and heating DSC curves shifted to higher temperatures with increasing concentration of both monovalent and divalent cations. With progressive addition of monovalent cations, the exothermic enthalpies gradually increased, and the exothermic peak for a mixture with sufficient monovalent cations became much sharper and this enthalpy significantly increased compared to that for a 0.3% GELL alone with the same concentration of monovalent cations. In the presence of divalent cations, the exothermic enthalpies for mixtures showed maximum at a certain concentration and then decreased with increasing concentration of divalent cations. In the presence of sufficient divalent cations, the cooling DSC curve for a mixture became much broader and this exothermic enthalpy significantly decreased compared to that for a 0.3% GELL alone with the same concentration of divalent cations. KGM is attached to the surface of large aggregates of GELL helices, and since cations promote GELL self-aggregation by the screening effects, the synergistic interaction between GELL and KGM was promoted with increasing concentration of cations. However, sufficient divalent cations formed various aggregates of GELL helices with different thermal stabilities, so that the phase-separation in GELL/KGM mixtures was promoted in the presence of sufficient divalent cations.

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